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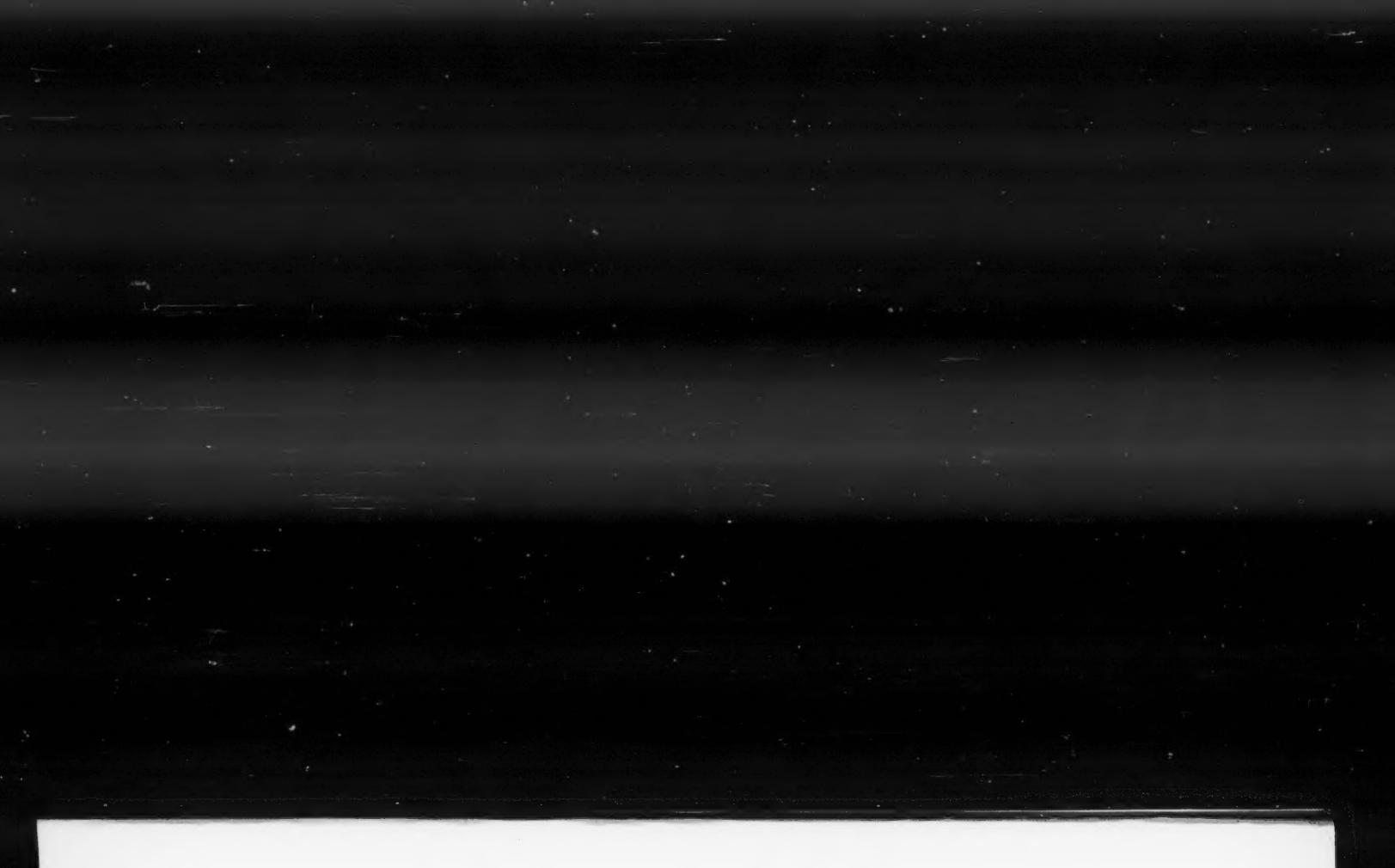

From the Department of Occupational Hygiene, the National Institute of Public Health, Tomteboda, Sweden.

A Method for Determination in Vivo of the Rate of Ciliary Beat and Mucous Flow in the Trachea.

By

TORE DALHAMN.

Received 6 July 1954.

The ciliary propulsion of secretion from the respiratory mucosa is one of the body's most important defence mechanisms against *inter alia* infections of the respiratory tract. Impairment of this mechanism, *e. g.* by irritating gases or dust, may therefore be expected to entail more or less deleterious consequences for the affected person.

In order to study the effect on mucous flow of low concentrations of some pulmonary-irritant gases common in industrial processes, I have attempted to analyze the changes thus brought about in ciliary function. For registration of the ciliary movements and estimation of the rate of mucous transport a special apparatus had to be constructed.

These two factors have earlier been studied separately both *in vivo* and *in vitro*, using different methods on several animal species (*e. g.* CRALLEY 1942, GRAY 1928, 1930, JENNISON and BUNKER 1934, LUCAS 1933, 35, LUCAS and DOUGLAS 1934, MARTIUS 1884 and PROETZ 1953). But the literature contains no report of systematic, simultaneous investigation of ciliary movement and mucous flow. Moreover, it would seem that very few experiments have been performed on mammals without saline solutions having been dropped onto the mucosa to prevent drying.

For study of the action of various gases and dusts a method has been evolved which permits *in vivo* experiments on mammals

(rats and guinea pigs) while avoiding all influence of foreign substances such as saline solutions. The method also permits simultaneous determination of the frequency of ciliary beat and the rate of mucous flow in the trachea, and detailed analysis of a single complete ciliary cycle. Variations can be brought about in the temperature and humidity of the tracheal environment and in the animals' body temperature.

Method.

The main components of the apparatus are a *moist chamber*, a *microscope*, two *cinematograph cameras* and *illumination devices*. In some cases stroboscopic determination was made of the speed of ciliary beat.

The moist chamber is provided with a water bath fitted with appliances for stirring and immersion heating. The temperature of the water, and consequently that of the air in the chamber, can be adjusted by a thermoregulator *via* the immersion heater. A contact thermometer and a contacting member connected to a rapidly-registering thermoelectric temperature indicator show the temperature inside the chamber.

The microscope is a Leitz Ultropak with a 5 × ocular and a 6.5 × objective.

One of the cameras is a Bell and Howell, type 70 S 16 mm, with a frequency of 128 exposures per second. The other is a Kodak Eastman High Speed camera 16 mm with a frequency of exposure variable from 500 to 3,000 per second. By placing a heat-reflecting filter in the path of the beam (interference filter Filtroflex type B), the increase in temperature when the Bell and Howell camera is in focus can be reduced to 0.2—0.3° C.

The stroboscope is of Drello type with an illumination range of 350 to 6,000 per minute. The source of light is a xenon discharge lamp.

Rats were anaesthetized with intraperitoneal avertin. The trachea was exposed and its soft parts incised by electrocoagulation, so that bleeding was avoided. It was then very easy to open the cartilaginous rings sufficiently to permit microscopy.

There was no risk of drying of the tracheal mucosa during this process, since no more than 25 seconds elapsed from its exposure until the animal was placed in the moist chamber. The temperature in the chamber was adjusted to 31—35° C and the relative humidity to 95—97 per cent.

The ciliary activity, expressed as beats per unit of time, was registered with the above-mentioned Bell and Howell camera (128 exposures per second) and calculated on subsequent "slowmotion" projection. The various phases of the ciliary cycle were studied by filming the light reflected by the cilia with the Kodak Eastman camera (approximately 500 exposures per second).

The rate of mucous flow was estimated by sifting finely pulverized charcoal through a filter web (mesh 0.08 mm, thread thickness 0.05 mm) over the trachea and, with a scale built into the ocular of the microscope, measuring the time required for a particle to move a certain distance. In many cases it was not necessary to use charcoal powder since shed particles of the mucous layer served as indicators.

Results.

In preliminary investigations with the above-described apparatus, with the humidity in the chamber and the animals' body temperature constant, the minute rate of ciliary beat in the great majority of cases ranged from 1,300 to 1,500 and that of tracheal mucous flow from 11 to 16 mm per minute.

The readings could be reproduced when the animals had lain in the chamber for as long as 80 minutes. Several animals remained in the chamber for more than two hours without apparent damage to the ciliary mechanism.

The influence on this mechanism of low concentrations of an irritant gas was tested in "acute" and "chronic" experiments. In the former, in which a stream of air of approximately 90 per cent relative humidity was directed onto the trachea, a concentration of 0.005 volumes per cent SO_2 appeared to be sufficient to arrest ciliary movement in less than one minute. This effect was reversible and could be reproduced many times. In the "chronic experiment" rats were exposed in a special chamber to SO_2 (0.001 volumes per cent) about 6 hours daily for eight weeks. At the end of this time it was observed that, although the rate of ciliary beat was still normal, many animals showed considerable retardation of mucous flow. In some there was no measurable flow. The mean rate for the others was 5.4 mm per minute. Microscopic analysis of the mucosa showed *inter alia* marked hyperplasia and in some cases loss of cilia. In most animals the lamina propria layer appeared to be richly vascularized and oedematous. These findings will be published in greater detail later.

Discussion.

In studying the ciliary function of mucous propulsion it is essential that the following factors at least be taken into consideration, viz. the temperature of the examined tissue, the humidity

of the environmental air, possible local irritative properties of substances used, and the viscosity and pH of the mucus. PROETZ (1933) described how, when the temperature of the mucosa was raised, the ciliary function gradually intensified, but decreased when the temperature was further raised and at 43 to 44° C finally ceased. The same writer also pointed out that "the only natural enemy known to cilia in their line of function is excessive drying". A few minutes' drying is sufficient to cause irretrievable destruction of the cilia.

That ciliary activity can be affected by various pharmacological substances is well known (PROETZ 1934). But not all workers would seem to be aware that ciliary activity may also be altered by various solutions of physiologic saline (LUCAS 1933).

In investigations of the rate of mucous flow it is probably extremely unphysiologic to drop saline solutions onto the membrane. Apart from a possible influence on ciliary activity, such a procedure may above all alter the viscosity of the secretion and thereby the functional environment of the cilia.

Measurement of the pH and viscosity of the tracheal mucus is made considerably difficult by the fact that the amount of secretion, even under favourable conditions, is extremely small. Moreover, the mucus is not homogeneous in viscosity, since it contains shed cells, etc.

With the method briefly described in this paper it is possible to eliminate three of the above-mentioned variables in investigations of ciliary function and mucous flow. The body temperature can be kept constant or can be varied within wide limits; and as the relative humidity of the chamber is about 95 to 97 per cent, it is unnecessary to apply saline solutions to prevent drying of the mucosa.

Changes in the pH of the mucus play an important part in ciliary function. In particular, increased concentration of hydrogen ions seems very rapidly to produce retardation of ciliary activity and therefore slowing of mucous flow (GRAY 1928). When the cilia are beating normally such slowing may conceivably be due to changes in the viscosity of the mucus.

Considering the long time the animals could remain in the moist chamber without apparent change in either the frequency of ciliary beat or the speed of mucous flow, any alterations in the pH and viscosity of the mucus in animals unexposed to respiratory irritants probably were so small as to lack major significance.

Determination of pH and viscosity was not included in the above-described method. In model experiments with animals exposed to respiratory irritants, however, I have attempted to obtain some idea of the changes in the mucous pH.

The apparatus used is thus well suited for study of the rate of ciliary beat and of mucous flow. The results obtained in rats exposed to SO_2 appear further to have demonstrated the usefulness of the method and strongly emphasized the importance of simultaneous determination of these two factors.

Summary.

A method is described by which simultaneous determination can be made of the rate of ciliary beat and that of the tracheal flow of mucus in small living mammals (rats and guinea pigs). The body temperature and the temperature and humidity of the environmental air can be varied. All influence of foreign substances such as saline solutions is avoided. The various phases of a single ciliary cycle are registered with a special camera. The method also permits study of the acute and chronic effects on the mucosa of different substances. Some experiments with SO_2 are briefly reported.

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The Use of a Compressed Air Operated Shock Tube for Physiological Blast Research.

By

HJÖRDIS CELANDER, CARL-JOHAN CLEMEDSON, ULF A. ERICSSON and HOLTER I:SON HULTMAN.

Received 22 July 1954.

When studying the mechanical and physiological effects of shock waves, it is desirable to produce a shock wave, the maximum pressure and impulse of which can be determined beforehand. Furthermore, it should preferably be possible to vary each of these quantities independently of each other.

In most studies in this field the required shock wave has been generated by means of detonating high explosive charges. In this way many investigations on the physiological effects of a varying maximum pressure have been performed (CLEMEDSON 1949). On the other hand, the conditions in this type of blast experiments are not favourable to the same extent, if one wishes to study the influence of a varying impulse at constant maximum pressure.

The scope of this paper is to give a general description of a compressed air operated shock tube, by means of which shock waves of different pressure and duration can be obtained, and of the arrangements for using the tube for biological blast injury experiments with mice as experimental animals. The shock tube method of inflicting blast injuries to experimental animals has been used earlier by CASSEN et coll. (1950, 1952). The shock tube to be described here is especially suited for studies of the effects of the impulse and the duration of the shock wave as these prop-

erties of the shock wave can, at a constant shock front pressure, be varied within rather broad limits. Furthermore, the shock wave obtained in the shock tube is more ideal for blast injury studies than a blast wave from a high explosive charge as it is plane and its velocity and front pressure cannot be considered to vary appreciably with distance.

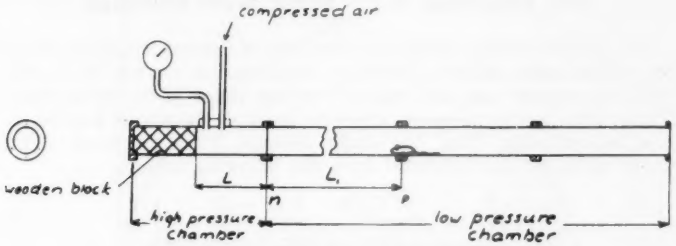

Fig. 1. Schematic diagram of the shock tube.

Description of the Shock Tube.

Figure 1 shows the general design of the shock tube. It consists of one high pressure chamber and one low pressure chamber separated from each other by a diaphragm of celluloid (M). The pressure in the high pressure part is increased by pumping compressed air into it by means of a motor-driven compressor. The low pressure part of the tube contains air of atmospheric pressure. When the pressure in the high pressure part has reached the breaking-point of the diaphragm, this bursts, and a shock wave is generated, the front of which travels at a certain velocity, W, into the low pressure chamber. The tube is made of 1 m long parts of boiler tubes, that are furnished with flanges and tightly screwed together. The tube has a circular section area, the diameter being 4 in. The section area of the shock tube has to be the same throughout its whole length, so that an one-dimensional shock wave can be produced.

The length of the high pressure chamber, L, which is maximum 1 m, can be varied by screwing tight-fitting wooden blocks of different length into the bottom end of the chamber. The length of the low pressure part of the tube, L_1, can be varied by removing or adding one or more tube parts. Its far end can be conveniently closed, if one wants to study the reflection of the shock wave.

The high pressure chamber is furnished with a manometer for the indication of the diaphragm bursting pressure. The wall of the low pressure chamber has some holes for the insertion of gauges for the measurement of shock wave pressure and duration.

The Properties of the Shock Wave Produced.

The qualities of the shock wave that are of interest when comparing the physiological effects of different shock waves are the front pressure, the impulse and the duration and the shape of the shock wave. These data can be measured experimentally or calculated and estimated theoretically. Thus, the static pressure, P, in the front of the shock wave can be calculated from the following formula:

$$\frac{P_o}{P_*} = \xi \left[1 - \frac{C_o (\varkappa - 1)(1 - \xi)}{C_* \sqrt{2\varkappa\xi \{\xi (\varkappa - 1) + \varkappa + 1\}}} \right]^{\frac{2\varkappa}{\varkappa - 1}} \qquad (1)$$

where P_o = the outside barometric pressure,
P_* = the pressure in the high pressure chamber, when the diaphragm bursts,
C_o = sound velocity in outside air,
C_* = sound velocity in the high pressure chamber when the diaphragm bursts. Usually $C_o = C_*$
$\varkappa$ = a gas constant (= 1.4 for air).
$\xi = \frac{P_o}{P}$, where
P = the total static pressure in the shock wave front, that is the pressure measured by a hypothetic manometer following the movement of the gas particles in the shock wave.

From the above formula, P_* can be solved but not ξ or P. For that reason a diagram (see fig. 2) has been drawn for P as a function of P_*.

Derivation of formula (1) would be outside the scope of this paper. For a more comprehensive study of the theoretical basis of the shock tube the reader is referred to the physical literature, *e. g.* to the paper of BLEAKNEY, WEIMER and FLETCHER (1949), where formulae for the static pressure and the velocity and Mach number of the shock wave front are found. Comprehensive treatises of the theoretical and practical aspects of air shock waves are given by COURANT and FRIEDRICHS (1949) and in the book Effects of Atomic Weapons (1950).

When the shock wave moves into the low pressure chamber a rarefaction wave simultaneously moves into the high pressure part. This wave is reflected against the bottom of the high pressure chamber and then travels back into the low pressure chamber. As the gas particles in the shock wave are moving in the same direction, the velocity of

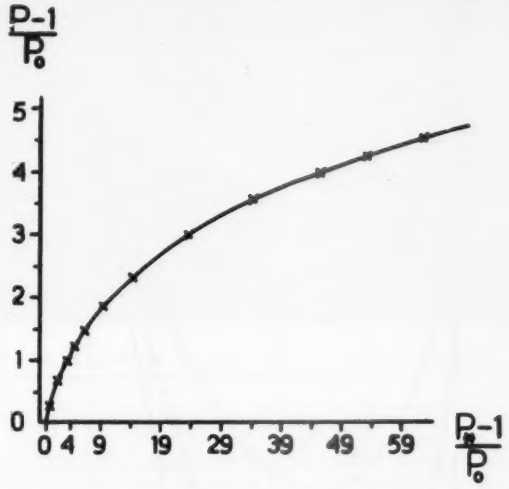

Fig. 2. Dimensionless static overpressure in the shock front, $\frac{P-1}{P_0}$, as function of the overpressure in the high pressure chamber, $\frac{P_*-1}{P_0}$.

the rarefaction wave will, thus, be further increased. Consequently, the rarefaction wave gradually reaches the front of the shock wave. From the above-mentioned it is evident, that the longer the high pressure chamber, the longer will be the duration, t, of the shock wave for one and the same distance from the diaphragm and for one and the same shock wave pressure. Fig. 3 shows the calculated duration, t, for different values of $\xi \left(= \frac{P_0}{P}\right)$, L and L_1 being the length of the high and low pressure part of the shock tube. For $\frac{L_1}{L} = 16$ the duration within the range $\xi = 0.40 - 0.56$ will be < 0, which means that in this case, the rarefaction wave has reached and weakened the shock wave (CELANDER 1952).

An important fact to notice is, that the product $P \cdot t$ does not give the whole impulse of the shock wave. This may be seen from figure 4 a and b, which shows a pressure gauge record and the corresponding constructed pressure-time curve with the *plateau duration* time, t, mentioned above and the *tail duration* time, t'. The product $P \cdot t$ corresponds to the dashed area in figure 4 b. Thus, the variation in t will be greater than that in the impulse. This is especially marked for short plateau durations.

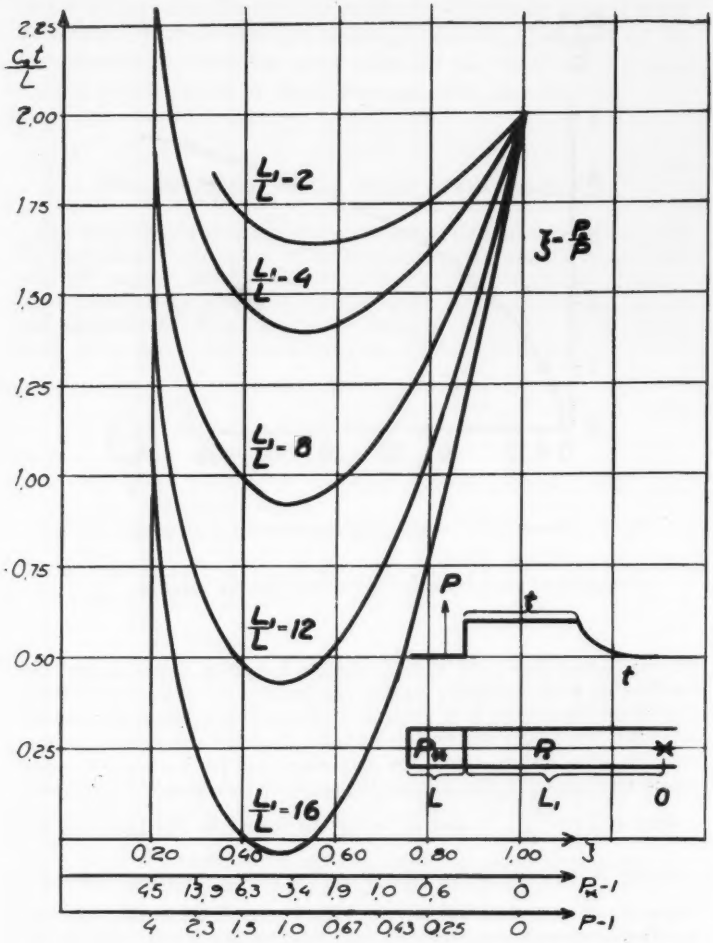

Fig. 3. Curves for obtaining the plateau duration time, t, for different values of $\bar{s}$ and $\frac{L_1}{L}$. Linear interpolation in the vertical direction. O = the exposed object (mouse). For explanation of symbols see text.

The static pressures and durations mentioned above refer to the ideal case with an undisturbed shock wave. Any object *e. g.* an experimental animal placed in the shock tube will, however, cause a stagnation and

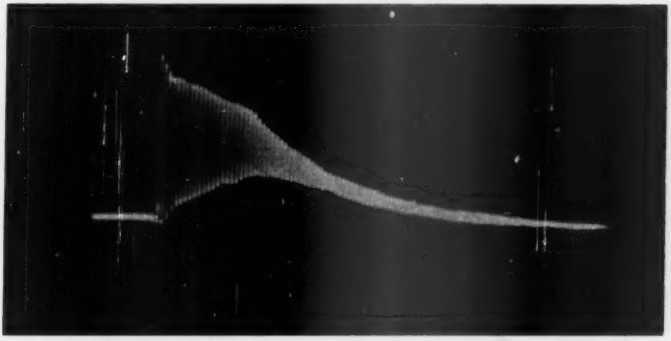

Fig. 4 a. A gauge record of a shock wave obtained in the shock tube.

reflection of the shock wave, the degree of which depends in a complicated way on the properties of the obstacle. The object will then receive a pressure that is greater than the static pressure, P. In order to show the limits for such increases in pressure caused by local stagnation of the gas particles, the reflection pressure, that is the pressure which the shock wave should exert against a plane disc at the bottom of the low pressure chamber, is also given.

The reflection pressure, P_2, can be calculated from the following formula:

$$\frac{P_2 - P_0}{P - P_0} = 1 + \frac{2\varkappa}{\xi(\varkappa + 1) + \varkappa - 1} \qquad (2)$$

where P_2 = the reflection pressure
P = the static shock wave pressure
P_0 = the outside barometric pressure, and
$\varkappa$ = a gas constant (= 1.4 for air)

Fig. 5 shows the reflection pressure, P_2, as a function of the diaphragm bursting pressure, P_*, in the high pressure chamber.

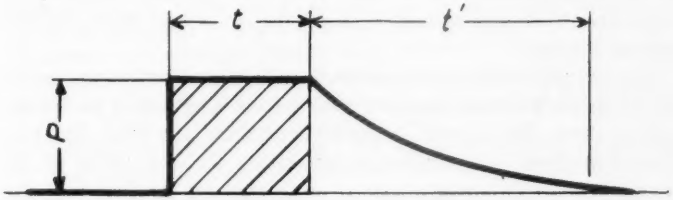

Fig. 4 b. The pressure-time curve, corresponding to the record in fig. 4 a.

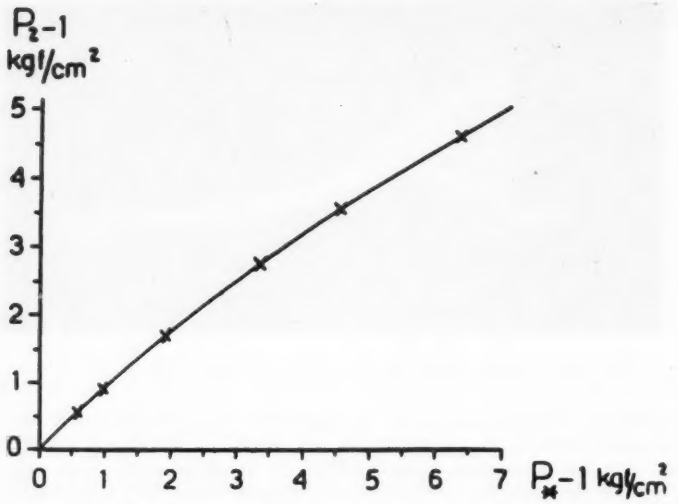

Fig. 5. Reflection overpressure, $P_2 - 1$, as a function of the overpressure in the high pressure chamber with $P_0 = 1$ kgf/cm².

Arrangements for Physiological Experiments.

The dimensions of our shock tube will not permit the use of experimental animals bigger than a mouse. Only one mouse can be exposed each time in order to cause the least possible disturbance of the shock wave. The animal can be placed at any desired point in the low pressure chamber of the shock tube. For most experiments it will be most suitable to place it at point P (see fig. 1) with its nose turned towards the high pressure chamber. In order to prevent the animal from moving from the place of exposure, it is enclosed in a small bag of coarse-spun, loosely woven fabric.

For the physiological experiments it has been found convenient to use a low pressure chamber with a total length of 5 m. Its far end is open. The animal is placed 2 m from the open end and thus 3 m from the diaphragm separating the two parts of the shock tube. This arrangement is motivated by the fact that the disturbances, which arise when the shock wave passes out through the open end of the low pressure chamber to expand in the open

air, will not have time to reach the animal before the whole shock wave has passed it.

The animal is subjected to an acceleration caused by the shock wave and is thrown out of the shock tube. To prevent its being injured by falling on to the floor, a loosely stretched sail is placed just behind the opening of the tube. The displacement of the animal sets in gradually, so that the shock wave will have passed and exerted its effect before the animal has been moved noticeably from its place of exposure.

The physical data determined in every experiment, are the diaphragm bursting pressure, P_*, in the high pressure chamber, the lengths, L and L_1, of the high and low pressure parts of the shock tube, and the air temperature. The last-mentioned is necessary to know for the calculation of the sound velocities C_o and C_*.

Summary.

The advantage of using a compressed air operated shock tube instead of blasting high explosive charges for certain types of physiological blast injury research is briefly discussed.

A description is given of a shock tube and of the special arrangements necessary for exposing mice to shock waves in that tube.

Some theoretical considerations concerning the shock tube and the data necessary for the calculation of the physical properties of the shock wave obtained are given.

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From the Research Institute of National Defense, Medical Department, Sundbyberg, Sweden, and Physical Department, Stockholm, Sweden.

A Study on the Relation between the Duration of a Shock Wave and the Severity of the Blast Injury Produced by It.

By

HJÖRDIS CELANDER, CARL-JOHAN CLEMEDSON, ULF A. ERICSSON and HOLTER I:SON HULTMAN.

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When dealing with the loading or injurious effects of a high explosive shock wave, there are mainly three qualities of the shock wave that are supposed to be of importance *viz.* the maximum or peak pressure, the momentum or impulse (*i. e.* the pressure-time integral) and the duration of the shock wave as an indication of the shape of the shock wave. As far as technical constructions *e. g.* buildings are concerned the relation between the qualities of the shock wave and its damaging properties is fairly well established.

In physiological experiments the significance of the maximum pressure and the impulse for the severity of the blast injury has been dealt with by some authors (see WAKELEY 1945 and CLEMEDSON 1949). In general, the maximum pressure will be the most important factor if the natural period of the exposed construction (tissue) is short as compared with the duration of the shock wave (see *e. g.* SCHARDIN 1950). If the natural period is long, the impulse essentially will determine the extent of damage. In the first case the construction (tissue) will have been loaded until rupture, before the blast pressure has fallen below the harmless level, whereas in the second case the shock wave has passed, before the tissue has reached its breaking deflection. Usually the conditions

will not be so simple and there will be a gradual transition between the two extremes.

From the definition of the impulse as the time integral of pressure it is evident, that even if the impulse values of the two shock waves are identical, the duration of the shock wave can nevertheless be different. Of the three qualities, the maximum pressure, the impulse and the duration of the shock wave, the last one has received the least attention when physiological effects of the shock wave are concerned. An investigation quoted by PENNEY and PIKE (1950) and which seems to be the only one of this kind in the literature, showed, however, that the duration was relatively unimportant provided it is short.

One reason why the correlation between the duration of the shock wave and the degree of blast injury has not been studied more in detail might be the difficulties to vary, within wide limits, the duration of the shock wave without changing the maximum pressure. Such variations of the duration great enough and at a constant blast pressure can hardly be produced by blasting high explosive charges in the open but it was found that they can be obtained in the shock tube described in a previous paper (CELANDER, CLEMEDSON, ERICSSON and HULTMAN 1954).

The present investigation was undertaken in order to study the injurious effects of shock waves of different durations. By changing the length of the high pressure chamber of the shock tube it has been possible to obtain shock waves, the duration of which differs by about a tenth power.

Procedure.

One hundred and forty-four white mice weighing between 17 and 24 g were used for this study. The shock tube used and the general arrangements of the experiments are described in our earlier paper. In order to obtain shock waves of various durations three different lengths, L, of the high pressure chamber of the shock tube were used. These lengths were 1.0 m, 0.52 m and 0.22 m. Four series of blastings with different thickness of the diaphragm were performed with each length of the high pressure chamber. The thicknesses of the diaphragms employed were 0.25, 0.375, 0.50 and 0.75 mm. With these diaphragms shock waves with a static overpressure of 1.0, 1.3, 1.6 and 2.0 kgf/cm^2, respectively, were obtained.

Animals that were not killed by the shock wave within one hour after the exposure were sacrificed after that time. The mortality rate given below, therefore, is the one hour mortality rate. All animals were

autopsied and their lungs removed and weighed separately. The extent of the lung hemorrhage was expressed by the quotients of hemorrhage $\frac{W_1}{W_0}$ according to CLEMEDSON (1949). In this expression W_1 denotes the weight of the blast damaged lung and W_0 the calculated normal weight of the same lung. This value is calculated from the body weight of the animal and the share of the lungs in the total body weight. From a material of 50 normal mice the mean values 0.19 and 0.41 per cent of the total body weight have been obtained for left and right lung, respectively.

Results and Discussion.

The animals exposed show various degrees of blast injuries, predominantly hemorrhages in the lungs. It was intended, at first, to use the quotients of lung hemorrhage as an objective criterion of the degree of injury. It was, however, on an early stage found, that the errors of these determinations due to the small size of the mouse lungs were too great to give a reliable index of the damaging effect of the shock wave. There existed, indeed, a clear difference between the groups, the quotients of hemorrhage being greater with increasing maximum pressure and longer duration of the shock wave, but the variations within the groups were too great to allow any definite conclusions to be drawn. Therefore, instead, the mortality rate of each group has been used as the sole measure of the effect of the shock wave. Of the animals that died of their injuries most died within the first 5 to 10 minutes after the exposure. The experimental data for the four different groups of experiments are tabulated in Table 1. The number of animals in each group usually is 10 to 15. One exception makes the group exposed to the highest max. pressure and the longest duration (see group IV in Table 1). As the animals in that group were instantly killed and heavily mutilated by the shock wave it seemed needless to expose more than a few animals in that group.

As seen from Table 1 it has been possible to maintain a constant static overpressure in the shock wave although its duration has been varied within rather wide limits. The duration of the shock wave is to a certain degree related to the maximum pressure, as it is evident from Table 1 that to a higher blast pressure corresponds a somewhat longer duration. This relation, however, is insignificant as compared with the very much greater differences in duration which are obtained by changing the length of the

Table I.

Tabulation of the experimental data.

Group	Length, L, of high pressure chamber in m	Qualities of the shock wave			No. of animals exposed	Dead	
		Static overpressure kgf/cm²	Reflection overpressure kgf/cm²	Duration millisec.		Number	Per cent
I	1.0	1.0	2.9	4.4	18	6	33
	0.52	1.0	2.9	1.9	10	2	20
	0.22	1.0	2.9	0.15 ± 20 %	10	0	0
II	1.0	1.3	4.0	4.6	10	10	100
	0.52	1.3	4.0	1.9	10	5	50
	0.22	1.3	4.0	0.15 ± 20 %	10	1	10
III	1.0	1.6	5.1	4.7	10	10	100
	0.52	1.6	5.1	2.0	15	13	87
	0.22	1.6	5.1	0.15 ± 20 %	15	11	73
IV	1.0	2.0	6.7	5.0	6	6	100
	0.52	2.0	6.7	2.1	15	14	93
	0.22	2.0	6.7	0.25 ± 20 %	15	12	80

high pressure part of the shock tube. Thus, it is seen from the table that at a static overpressure of, for example, 2.0 kgf/cm² and a length of the high pressure chamber of 1.0 m, the duration of the shock wave is about 5 millisec., whereas with a chamber length of 0.22 m the duration is only 0.25 millisec.

The table shows that the number of lethal injuries grows rapidly with increasing shock wave pressure. It is also evident that within groups with equal pressure the mortality is greater the longer the duration of the shock wave. It is of special interest to notice, that the correlation between duration and mortality is especially pronounced in the group exposed to a moderate shock wave pressure (group II) but that it is much less if the pressure is lower (I) or higher (III and IV).

It is evident from the present investigation that besides the maximum pressure also the duration of the shock wave is of importance for the production of blast injury. The most important factor, however, is the maximum pressure. The effect of duration will assert itself the most in cases where the moderate maximum pressure per se would only cause a moderate blast injury.

Summary.

The injurious effect of shock waves of various durations was studied on mice exposed to shock waves in a compressed air operated shock tube.

It was found that the longer the duration of the shock wave the more severe was the injury inflicted. The influence of the duration is most pronounced in cases where an only moderate maximum pressure per se would cause only a moderate injury. Of the two factors, the maximum pressure and the duration, the first one is the most important as far as injurious effect is concerned.

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Effects of Intrathecal Injections of Adrenaline and Noradrenaline on Blood Sugar and Blood Pressure in the Cat.

By

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BECHT (1920), HELLER (1933) and LEIMDORFER (1950) have reported that large doses of adrenaline injected intrathecally into cats or dogs do not raise the blood pressure, whereas the same dose administered intravenously to the same animal produces a marked rise. LEIMDORFER, ARANA and HACH (1947), working on cats, have shown that intracisternal injection of adrenaline results in hyperglycemia, the concentration and duration of which is dependent on the size of the dose. As the blood pressure and heart rate were not affected, LEIMDORFER expressed the view that the hyperglycemia was brought about by a direct central action of the adrenaline.

He also found that intracisternal injections of noradrenaline (75 μg—375 μg per kg body weight) in dogs produced a rise in the blood sugar concentration, but that in larger doses there is a concurrent rise in blood pressure, tachycardia, and arrhythmia. Adrenaline (0.5—1.5 mg per kg body weight) did not affect the blood pressure or heart rate, whereas the blood sugar level showed a marked rise. In both cases analgesia, sleep, and anesthesia were produced, which LEIMDORFER considered to be due to a central action.

In the present investigation the effects of noradrenaline, and in some animals of adrenaline, on the blood glucose and blood pressure in cats have been studied after injection into the cisterna magna, the third ventricle, and during continuous intravenous infusion.

Cats of both sexes were used in the experiments, their weights varying between 2.8 and 5.4 kg. All the animals fasted for 18 hours before the experiments, but were allowed to drink water. The blood sugar concentration was determined in arterial blood ad modum Folin. The animals were anesthetized by intraperitoneal injection of Nembutal (Abbott). Synthetic l-adrenaline and l-noradrenaline were used and the amounts expressed in terms of the hydrochlorides.

A. The Influence on the Blood Sugar Level and Blood Pressure of Intracisternal Injection of 50 μg of Noradrenaline and 50 μg of Adrenaline per kg Body Weight.

Cannulas with a calibre of 0.45 mm were used for puncture of the cisterna magna. The needle was left in position throughout the experiment. The dose was given in 0.09—0.12 ml of saline after aspiration of an equal volume of cisternal fluid. The blood pressure was registered from the left femoral artery, and blood taken for determination of the blood sugar level from a poly-ethylene catheter in the right femoral artery. After cisternal puncture the animal was heparinized. Noradrenaline was injected 30 minutes after puncture of the cisterna magna to obviate any possible mechanical effect on the blood sugar due to the puncture. Blood pressure and blood sugar were registered during a period of 3—6 hours. At the conclusion of the experiment 0.05 ml fluid was aspirated and injected intravenously, and its effect on blood pressure studied. When the experiment was finished, 0.05 ml of a methylene blue solution was injected via the needle to localize its position and study the diffusion in the ventricular system. After freezing, the cat's head was sawn in half and the distribution of the methylene blue studied.

Results.

Injection of methylene blue showed, in 4 animals, spread into the subdural space round the cerebellum and the underside of the hypophysis. In no animal were there signs of spread into the third or fourth ventricle. Clear fluid was obtained by puncture.

After injection of noradrenaline the blood sugar level rose in these cases by 22—40 mg%, reaching the peak after 30 minutes and returning to the original level after $1^1/_2$—2 hours. Intravenous injection of 0.05 ml fluid after $3^1/_2$ hours had elapsed resulted in a rise in blood pressure of 40 mm Hg, which implies that even after this interval the noradrenaline was still present in high concentration. Throughout the experiment the variation in blood pressure did not exceed 20 mm Hg.

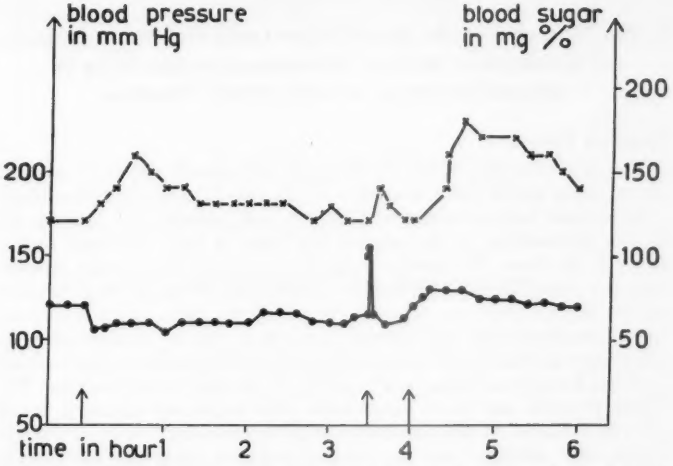

Fig. 1. The influence on the blood sugar level and blood pressure of intracisternal injection of noradrenaline and adrenaline.

× —— Blood sugar
● —— Blood pressure

50 μg noradrenaline kg/i.c. 0.05 ml cisternal fluid i.v. 50 μg adrenaline/kg i.c.

Two animals were given adrenaline in a dose of 50 μg per kg body weight after an interval of $3^1/_2$ to 4 hours, when the blood sugar was at its original level. This brought about a rise of 50—60 mg% in the sugar level, the peak being reached 30 minutes after injection, while the variations in blood pressure did not exceed 15 mm Hg. After a further 2 hours the blood sugar level was still raised.

In 2 animals a lesion occurred in the cerebral substance at the fourth ventricle. The fluid obtained was then slightly admixed with blood. In both cases a rise of 70—90 mg% in the blood sugar occurred before the injection of noradrenaline. 50 μg noradrenaline per kg body weight produced, in addition to the rise in the blood sugar, a marked increase in blood pressure in one of the animals, probably due to increased resorption, whereas in the other animal the blood pressure was not affected though the concentration of sugar in the blood rose from 200 to 252 mg%, falling again after 1 hour.

B. The Influence on the Blood Sugar Level and Blood Pressure of Injections of 50 μg of Noradrenaline and 50 μg of Adrenaline per kg into the Third Ventricle.

Operative Procedure.

The skin over the vertex of the skull was incised and the bone dissected clean round the intersection of the coronal and sagittal sutures. A cannula with a calibre of 0.45 mm was introduced 2 mm caudal to the intersection in the median line, after a hole had been drilled through the bone. The needle was held in position by a collar screwed into the bone (DUNÉR, 1953). The needle was advanced to a distance of 19—20 mm from the external surface of the bone, and left in position throughout the experiment. 0.10—0.12 ml of noradrenaline or adrenaline in saline was injected 30 minutes after puncture. In 2 animals 0.05 ml Ringer's solution was injected 15 minutes after puncture. The blood pressure and blood sugar level were registered during a period of 3 to 6 hours. At the conclusion of the experiment 0.05 ml of a methylene blue solution was injected in order to study the diffusion in the ventricular system. The head was then frozen and sawn in half.

Results.

Injection of a methylene blue solution showed in 3 animals diffusion into the lateral ventricles, the fourth ventricle, and the subdural space round the cerebellum. No signs of cerebral lesion or hemorrhage were found. In 2 cases the fluid obtained by puncture was clear. 50 μg of noradrenaline per kg body weight produced a moderate rise in the blood sugar level of 30—45 mg% in every animal, the peak being reached after 30 minutes, and returning to the original level after $1^1/_2$ to 2 hours.

Immediately after the injection the blood pressure in 2 animals fell 10—15 mm, returning to its original value after 10 to 15 minutes, after which it remained constant.

Injection of 0.05 ml of Ringer's solution failed to produce any effect on blood pressure or blood sugar level.

Injection of 50 μg adrenaline per kg body weight after an interval of 3 hours produced in 2 animals a rise in the blood sugar level of 55—60 mg%, the maximum value being registered after 30 minutes. Two hours after the injection both animals showed a raised blood sugar concentration. Blood pressure variations did not exceed 15 mm Hg during the entire period of the experiment.

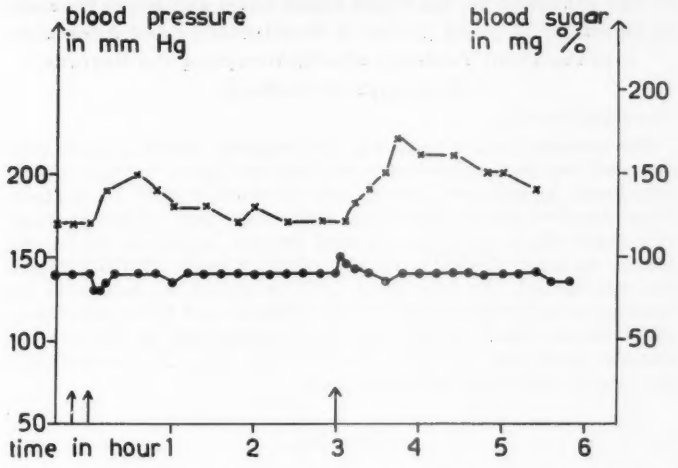

Fig. 2. The influence on the blood sugar level and blood pressure of injections of noradrenaline and adrenaline into the third ventricle.

× —— Blood sugar
● —— Blood pressure

50 μg noradrenaline/kg into the third ventricle

50 μg adrenaline/kg into the third ventricle

0.05 ml of physiological saline into the third ventricle

Hemorrhage in the ventricular system occurred in 2 animals; prior to injection the blood sugar level had shown a rise of 63 and 70 mg% respectively in these two cases. 50 μg noradrenaline per kg body weight in these animals produced a rise in blood sugar of 30 and 40 mg% respectively, and an increase in blood pressure of 20—30 mm Hg suggesting an increased resorption.

In one case the injection of methylene blue showed subarachnoidal diffusion; there were no signs of spread in the ventricular system. In this animal the blood sugar concentration rose from 120 to 205 mg% after injection of noradrenaline, the peak being reached after 30 minutes. The blood pressure remained unchanged for 2 hours, after which it fell successively.

C. The Influence on the Blood Sugar Level and Blood Pressure of Injection of 50 μg per kg of Noradrenaline and Adrenaline into the Third Ventricle after Interrupting the Nervous Pathways in the Neck.

Operative Procedure.

The external jugular veins and the common carotid arteries were dissected free, both sympathetics and vagi cut, and a tracheal cannula introduced. A needle was inserted into the third ventricle by the technique described above. The tissues in the neck were crushed by means of a chain which was tightened until pressure applied to the carotid arteries no longer effected a change in blood pressure. Artificial respiration was applied. The experiment could be started one hour after the crushing, as an initial increase in blood glucose and fall in blood pressure occurred. After one hour the blood pressure was 90—95 mm Hg, and the blood sugar concentration 150—160 mg%. The circulation in the arteries and veins was satisfactory.

Results.

Injection of a methylene blue solution showed diffusion throughout the ventricular system in 3 animals, without any sign of hemorrhage. The injection of 50 μg noradrenaline per kg body

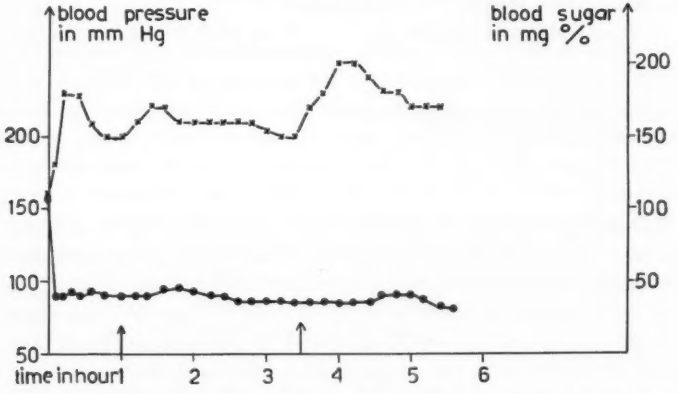

Fig. 3. The influence on the blood sugar level and blood pressure of injection of noradrenaline and adrenaline into the third ventricle after interrupting the nervous pathways in the neck.

× —— Blood sugar

● —— Blood pressure

50 μg noradrenaline/kg into the third ventricle

50 μg adrenaline/kg into the third ventricle

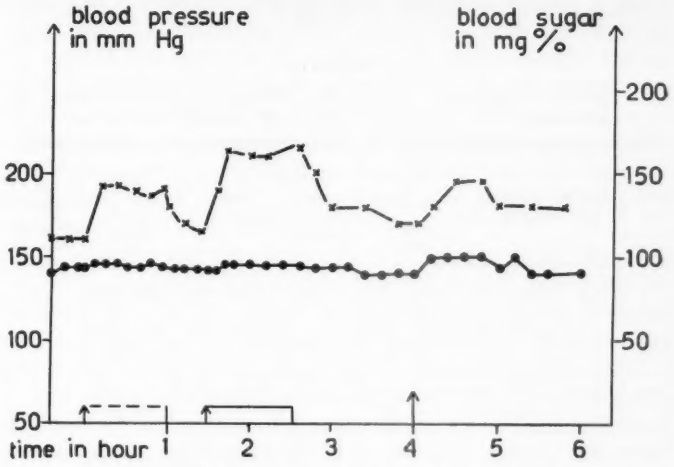

Fig. 4. The influence on the blood sugar level and blood pressure of continuous infusion of noradrenaline and adrenaline.

× —— Blood sugar
● —— Blood pressure

0.17 μg/kg/min. noradrenaline i.v.
0.032 μg/kg/min. adrenaline i.v.
50 μg/kg noradrenaline i.c.

weight into the third ventricle produced an increase in blood sugar of 22—31 mg%, the maximum value being registered after 30 minutes, after which the level remained constant for $1^1/_2$ to 2 hours in 2 animals. The blood pressure was between 90—85 mm Hg during a period of $2^1/_2$ hours, after which it fell. 50 μg adrenaline per kg body weight in one animal caused a rise in blood sugar of 55 mg% after 30 minutes, the level still being raised after $1^1/_2$ hours. Blood pressure was between 80—90 mm Hg.

D. The Influence on the Blood Sugar Level and Blood Pressure of Continuous Infusion of Noradrenaline and Adrenaline.

The infusion was continuous via a catheter in the femoral vein. The solutions contained 3 μg noradrenaline and 0.3 μg adrenaline per ml, respectively, in saline.

The rate of infusion was varied so as not to change the blood pressure. Cisternal puncture was performed in 2 animals when the infusion was discontinued. The experiments were carried out on 3 animals.

The results are presented in Table I.

Table I.

The effect of continuous intravenous infusion of noradrenaline and adrenaline on blood sugar and blood pressure in the cat.

Cat no.	Weight kg	Noradr. μg/kg/min.	Effect on blood sugar mg%	Effect on blood pressure	Adr. μg/kg/min.	Effect on blood sugar	Effect on blood pressure mm Hg
1	5.4	0.05 30'	128—170 (+ 42)	0	0.009 30'	130—165 (+ 35)	0
		0.09 30'	130—185 (+ 55)		0.025 30'	135—205 (+ 70)	
2	4.6	0.17 60'	108—140 (+ 32)	0	0.032 60'	115—165 (+ 50)	+5
3	4.5				0.017 30'	110—148 (+ 38)	0
					0.032 30'	110—170 (+ 60)	+5

Fifty μg noradrenaline (animal no. 2) or 50 μg adrenaline (animal no. 3) administered intracisternally raised the blood sugar 24 and 49 mg% respectively without raising the blood pressure.

Discussion.

Since LEIMDORFER in his experiments did not find any effect on the blood pressure after intrathecal injection of adrenaline and as the cerebro-spinal fluid still contained adrenaline after 5 to 6 hours, he believed that the adrenaline did not cross the barrier between the cerebrospinal fluid and the blood. He considered the resulting hyperglycemia to be brought about by direct central action.

The slow resorption from the dural space may be partly due to vasoconstriction caused by the high concentrations of the pressor amines.

FORBES, FINLEY and NARVA (1933) studied the reaction of the pial vessels to local application of adrenaline. Large arteries were affected in 72 per cent of cases, smaller ones in 29 per cent, while the veins and small veins showed constriction in 85 per cent of

cases. The venous constriction, then, was more marked in every experiment. In comparing these figures with those registered in the skin of cats, these authors found the constriction of the arteries in the skin to be 4 times as great as that in the pial arteries after an equally large dose of adrenaline.

Fog (1939) also studied the effect on arteries in the pia of a direct application of adrenaline. He reported that the larger arteries show slight constriction, whereas the small ones are less readily affected.

The analgetic and anesthetic effects of large doses of adrenaline (0.5—1.5 mg per kg) and noradrenaline (75—375 μg per kg) intracisternally in dogs reported by Leimdorfer, may well be the results of anoxia. In monkeys, Wu, Harnagel, Brizzee and Smith (1954) showed that intrathecal doses of adrenaline (0.6—1.0 mg per kg) caused paresis, decreased sensibility, and rigidity of transitory nature. The clinical picture resembled that associated with anoxia in the spinal cord. It is well-known that a reduction in blood circulation resulting from a mild vaso-constriction of arteries and veins has more far-reaching effects in the cerebral tissues than in tissues with lower metabolism.

Injections of 1 mg dl-noradrenaline intrathecally in man (Euler and Liljedahl, 1954) did not affect the blood pressure, and no anesthetic effect of this dose was observed. Analysis of cerebrospinal fluid and urine showed that the noradrenaline is slowly resorbed from the dural space and excreted in the urine.

In the experiments reported above, intracisternal injection of noradrenaline (50 μg per kg) produced a moderate rise in the blood sugar level without affecting the blood pressure; equal doses of adrenaline caused a more marked increase in the concentration of sugar in the blood without raising the blood pressure. Injections of equal volumes of noradrenaline and adrenaline into the third ventricle before and after interruption of the nervous pathways produced similar effects. Continuous intravenous infusion of noradrenaline and adrenaline in doses of 0.09 and 0.009 μg/kg/min., respectively, resulted in a rise in the blood sugar level corresponding to that caused by intracisternal injection, without affecting the blood pressure. Adrenaline thus had an effect on the concentration of glucose in the blood 5 to 10 times greater than that of noradrenaline, an observation which fully agrees with Schümann's report of 1948.

These experiments suggest that the hyperglycemic effect of injections of noradrenaline and adrenaline into the cisterna magna or the third ventricle is brought about by resorption from the dural space. The dose resorbed in a minute has been seen to be sufficient to raise the concentration of glucose in the blood in anesthetized cats without affecting the blood pressure.

Summary.

A rise of blood sugar was observed after 50 μg noradrenaline per kg body weight injected into the cisterna magna (22—40 mg%) or the third ventricle (30—45 mg%) in anesthetized cats.

The effects of the same dose of adrenaline was 50—60 mg% and 54—66 mg% respectively. These effects were obtained even after interruption of the nervous pathways in the neck. No effects were noted on the blood pressure.

Intravenous injection of 0.05 ml of spinal fluid 3½ hours after intrathecal injection of 50 μg noradrenaline raised the blood pressure 40 mm Hg.

In cases where lesions and hemorrhage occurred, a rise in blood pressure was observed after 50 μg noradrenaline intracisternally, suggesting a more rapid resorption. A rise in blood sugar was also noted in these cases prior to the injection.

Continuous intravenous infusion of 0.05—0.17 μg/kg/min. noradrenaline and 0.009—0.032 μg/kg/min. adrenaline, caused a rise in the blood sugar level of 32—55 mg% and 35—70 mg% respectively. The rise was of the same degree as injection into the cisterna magna or the third ventricle, without affecting the blood pressure.

The effects observed may be accounted for by resorption of small quantities from the dural space.

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The Cerebral Blood Flow in Man Determined by the Use of Radioactive Krypton.

By

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The first dependable quantitative determinations of cerebral blood flow in man were published in 1945 by KETY and SCHMIDT who devised the nitrous oxide method. In 1947 GIBBS, MAXWELL, and GIBBS described a method employing Evans' blue dye. Only the Kety—Schmidt method has been applied to any extent. In their first paper in the nitrous oxide method, KETY and SCHMIDT (1945) suggested that the similar use of an inert radioactive gas would offer certain advantages compared with nitrous oxide. A method employing the radioactive isotope krypton 85 (Kr^{85}) has been devised and is described here. It is based, as was the original nitrous oxide method, on the Fick principle (1870).

Methods.

The subject inhaled Kr^{85} from a closed respiratory system with circulating air for 14 minutes. Kr^{85} is a relatively weak beta-ray emitter (maximal energy 0.7 MEV) with no gamma-ray emission. The half-life is 12.9 years. In our experiments, the effective biological half-life was approximately 11 minutes. Kr^{85} was delivered in gas ampoules in a mixture of stabile inert gases. Small amounts of the gas mixture were transferred to the respiratory system (fig. 1).

Measurements of the concentration of Kr^{85} in the respiratory system were made by means of a Geiger-Müller tube (fig. 1, G), and a suitable concentration was maintained. Kr^{85} was not added during the actual examination.

The respiratory system (fig. 2) contained 60 liters of atmospheric air and approximately 6 mC Kr^{85}. A spirometer, a carbon-dioxide absorber, and a blower were inserted in the system. During the examination, oxygen was supplied in amounts varying with the subject's uptake in the following way (fig. 2): When the spirometer fell below a certain level, a contact closed a circuit which opened a valve, and oxygen passed into the respiratory system via a wet gas meter. By means of this gas meter, the total body oxygen uptake was measured

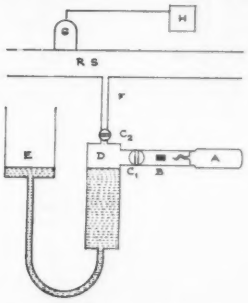

Fig. 1. Apparatus for the transfer of Kr^{85} to the respiratory system. A is the ampoule containing Kr^{85}, B is a metal anchor for opening the ampoule, C_1 and C_2 indicate cocks. By lowering the levelling bottle E when C_1 is open, Kr^{85} enters D, C_1 is now closed, C_2 opened and by raising E a suitable amount of activity is transferred to the respiratory system RS via F. G is a Geiger-Müller tube in connection with a counting ratemeter.

during the examination. The oxygen concentration of the circulating air was measured by volumetric gas analysis and was maintained between 20 and 23 per cent. For the first minute after the cessation of the experiment, the expiratory air was led out of the window in order to avoid contamination of the air in the laboratory.

During the examination, the subject was recumbent. After local anesthesia, needles with three-way stop-cocks were placed in the superior bulb of the left internal jugular vein and in the left femoral artery. Free passage through the cocks and cannulae during the procedure was assured by frequent flushing with dilute heparin saline solution. Mean arterial and mean venous blood pressures were followed. Coagulation of the blood was avoided by filling the dead space of the sampling syringes with a 30 per cent sodium citrate solution. Immediately prior to taking a sample, 2 cc of blood were withdrawn in order to clear the system. At short intervals (0.5—4 min.) during the fourteen minutes of Kr^{85} inhalation, 8 samples were taken from the artery and 8 from the vein.

Each sampling took 10 seconds, and 3—4 cc were taken in each syringe.

After 3 minutes, samples were taken for the determination of pH, oxygen saturation, carbon dioxide, and hemoglobin content. The

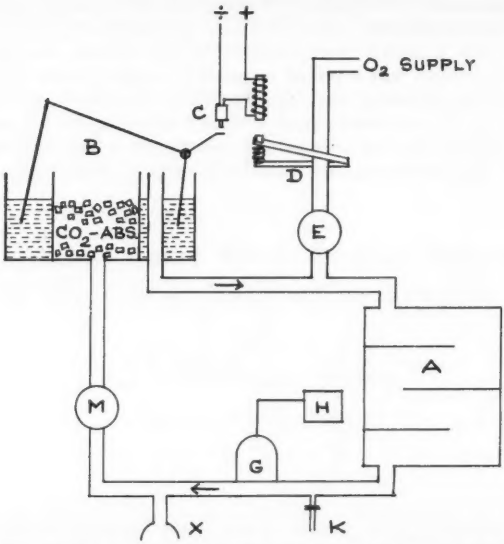

Fig. 2. The respiratory system. A is a tight container (40 liters), B a spirometer (20 liters) with carbon dioxide absorber, M a blower, X a mouthpiece for the subject, K a tube for Kr^{85} supply. G is a Geiger-Müller tube in connection with a counting ratemeter. Oxygen supply: when B falls below a certain level contact C closes a circuit opening valve D, and oxygen passes into the respiratory system via a wet gas meter E.

samples were kept at 37° C and analysed within half an hour. pH was measured with a glass electrode; the oxygen saturation was determined photometrically with Brinkman's hemoreflector; the carbon dioxide content was determined volumetrically; and the hemoglobin percentage was determined photometrically (standardised so that 100 per cent hemoglobin corresponded to an oxygen capacity of 18.5 volume per cent).

Special cuvettes were used for the determination of the relative concentrations of Kr^{85} in the blood samples (fig. 3).

Each cuvette consisted of a steel ring fitted with lateral tubes, an upper and lower mica window, and an upper and lower 0.1 mm thick protecting ring for fastening the mica windows. The steel ring was 3 mm high with an inner diameter of 25 mm and an outer diameter of 30 mm. The thickness of the mica windows corresponded to 4—4.5 mg/cm^2. The total mass of the mica windows did not vary more than $\pm$ 3 per cent from cuvette to cuvette. The components were fitted together at room temperature with "Araldite type 101".

After the plasma and blood corpuscles had been mixed, the blood was transferred from the syringe to the cuvette, which was sealed under slight positive pressure (fig. 4).

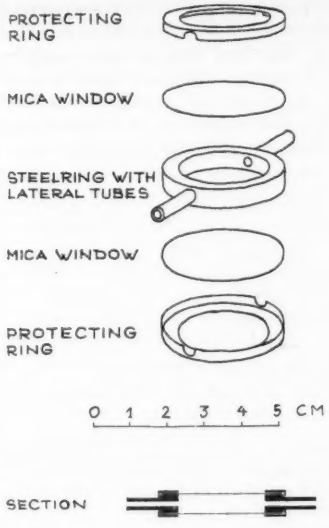

Fig. 3. The components of a cuvette.

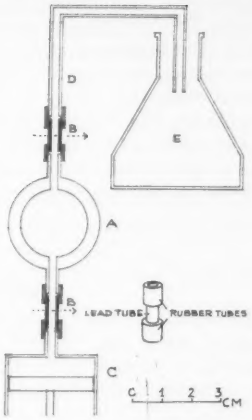

Fig. 4. Filling a cuvette with blood. The cuvette A is fitted with lead-rubber tubes B and is hereby connected to a syringe C and a pipe D. E is a flask for excess blood. The blood is syringed through the tubes and cuvette. The lead sections of the tubes are cut with pliers at the arrows, and the cuvette is hereby sealed.

The total volume of blood per cuvette was without importance, as the counting was performed on infinitely thick layers. Twenty Kr^{85} analyses were made for each determination of the cerebral blood flow, as duplicate analyses were made on the last two samples from each vessel.

The sealed cuvettes were placed in sockets in a horizontal plate (fig. 5) suspended between two pairs of lead-screened end-window Geiger-Müller tubes (diameter of end-window 25 mm). The two Geiger-Müller tubes of a pair had the same working tension and were both in connection with the same high voltage source and scaling instrument.

Each sample was counted for 15 minutes, 7 $^1/_2$ minutes by each pair of Geiger-Müller tubes. The device permitted automatic analysis of 18 samples under identical conditions in 135 minutes.

Employing the Kr^{85} concentration in question, about 7,000 emissions were recorded from the most active samples in 15 minutes. The cuvettes were standardised with blood taken from the artery immediately before the end of an examination. Cuvettes which deviated more than $\pm$ 1 per cent from the mean value of the observations after repeated standardisation were discarded. The standard deviation of the individual Kr^{85} analysis was therefore 1.6 per cent if 7,000 emissions or more were recorded.

As the solubility of Kr^{85} is lower in plasma than in red blood cor-

puscles (see supplement I), sedimentation may affect the counting results. The filled cuvettes were placed on the horizontal disc 30 minutes before the beginning of the counting. The first two or three samples were recounted after 2—3 hours, and the recounts were always consistent with the values first obtained, indicating constant counting conditions. The result of each analysis was expressed as a percentage of the most active sample, and plotted against the sampling times. The graphs obtained represent the variations in relative Kr^{85} concentrations in the two vessels.

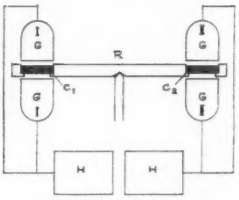

Fig. 5. Two cuvettes, C_1 and C_2, are placed in sockets in the horizontal plate R. I and II are two pairs of Geiger-Müller tubes, fixed above and below the plate. Each pair of Geiger-Müller tubes is connected to an amplifying and scaling instrument H.

Calculation of the Cerebral Blood Flow (CBF).

When calculating the CBF from the graphs representing the relative concentrations of Kr^{85} in the femoral artery and in the superior bulb of one internal jugular vein it is assumed:

I) that the blood in the femoral artery is representative of the arterial inflow in the brain.

II) that the blood in the superior bulb of one of the internal jugular veins is representative of the venous outflow from the brain (KETY and SCHMIDT 1948a).

III) that the CBF is constant during the observation period. (The results of repeated examinations with an interval of 30 minutes reported in this paper indicate that this is usually the case.)

IV) that the uptake of Kr^{85} in brain, plasma, and red blood corpuscles, like the uptake of nitrous oxide (KETY, HARMEL, BROOMELL, and RHODE 1948), is passive, *i. e.* it is governed by diffusion and solubility, and that the solubility does not differ from subject to subject or from higher animals to man. The partition coefficient brain: blood (S) for Kr^{85} was determined experimentally and was found to vary with the hematocrit value (see supplement I).

V) that the relative concentrations of Kr^{85} for the two vessels in an experiment of infinite duration can be calculated from the observations made during an experiment lasting 14 minutes. When using a closed respiratory system of 60 liters it was found that the arterial and venous curves between the 6th—9th minute and the end of the experiment (14th minute) could be expressed analytically in simple terms (see equations (3) and (4)). It is assumed that these

equations can be applied after the 14th minute. In five experiments, the observation period was extended to 20 minutes.

By using the Fick principle, the rate of uptake of Kr^{85} in the brain can be expressed by making the assumptions I), II), and III):

$$\frac{dC_b}{dt} = \frac{CBF}{100} [C_a(t) - C_v(t + \tau)] \ldots\ldots\ldots\ldots \quad (1)$$

where:

$C_b(t)$ is the concentration of Kr^{85} in the brain tissue at the time t, expressed in units Kr^{85} per g brain tissue. The brain tissue is defined to include the blood in the cerebral vessels.

CBF is the cerebral blood flow in cc blood per 100 g brain tissue per minute.

$C_a(t)$ and $C_v(t + \tau)$ are the concentrations of Kr^{85} in the blood of the femoral artery and in the superior bulb of one of the internal jugular veins respectively at the time t and $t + \tau$, where τ is the mean circulation time through the brain. The concentrations are expressed in units Kr^{85} per cc blood.

The solution of (1) is:

$$C_b(t) = \frac{CBF}{100} \int_0^t [C_a(t) - C_v(t + \tau)]\, dt \ldots\ldots\ldots \quad (2)$$

$C_a(t)$ and $C_v(t)$ can be expressed analytically for $t > 6$ to 9 minutes (see assumption V and fig. 6):

$$6\text{—}9 \text{ min.} < t < 14 \text{ min.} \quad C_a(t) = C_a(14) \ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

$$C_v(t) = C_a(14)\ [1 - k_1 e^{-k_2 t}] \ldots\ldots\ldots \quad (4)$$

where k_1 and k_2 are constants which can be determined by plotting $C_a(14) - C_v(t)$ on a semilogarithmic scale. By assuming (assumption V) the relations (3) and (4) to be valid even after the 14th minute formula (2) can be expressed:

$$C_b(\infty) = \frac{CBF}{100} \left\{ \int_0^{14} [C_a(t) - C_v(t + \tau)]\, dt + \int_{14}^{\infty} [C_a(14) - C_a(14)\ (1 - k_1 e^{-k_2 (t + \tau)})]\, dt \right\} \ldots\ldots\ldots \quad (5)$$

By inserting $C_b(\infty) = S\ C_v(\infty)$ (see assumption IV and supplement I) and rearranging, formula (5) is changed to:

$$CBF = \frac{100\ S\ C_a(14)}{\int_0^{14} [C_a(t) - C_v(t + \tau)]\, dt + [C_a(14) - C_v(14 + \tau)]\ 1/k_2}$$

$$\text{cc}/100\ \text{g/min} \ldots\ldots\ldots\ldots\ldots\ldots \quad (6)$$

The procedure for determining the right-hand side of equation (6) is as follows:

S is known (see supplement I).

$C_a(14)$ and $C_v(14 + \tau)$ are unknown, but relative measures are obtained from the graphs, and as $C_a(t)$ and $C_v(t + \tau)$ are expressed

in the same relative units it follows from (6) that the result is unaffected.

τ is the mean circulation time through the brain. It was determined as the time interval between the appearance of radioactivity in the arterial blood (0.07 to 0.10 minutes after the beginning of inhalation) and the time at which the venous curve started as determined by retrograde extrapolation by use of the three first venous samples. τ was found to be approximately 0.20 minutes for values of CBF between 35 and 60 cc/100 g/min., approximately 0.25 minutes for values of CBF lower than 35 cc/100 g/min., and approximately 0.15 minutes for values of CBF higher than 60 cc/100 g/min. These values for τ have been used for all the calculations.

k_2 is determined by semilogarithmic resolution of the difference between the arterial and venous curves for $t > 6$ to 9 minutes.

$\int_0^{14} [C_a(t) - C_v(t+\tau)]\,dt$ is the area between the arterial and venous curves (after displacing the latter τ minutes to the left). The area is measured by the trapezoid rule for each minute, but in the first minute from 0.1 to 0.5 minute and from 0.5 to 1.0 minute separately.

The extrapolation for time above 14 minutes (assumption V) did not increase the standard deviation of the calculated value for the cerebral blood flow significantly, as the standard deviation of the CBF (see the experiments referred to in the first part of the results) was 1.6 cc/100 g/min. when using formula (6) and 1.2 cc/100 g/min. when using the Kety—Schmidt formula (Kety and Schmidt 1948a), in which no extrapolation is made, on the same experiments. The extrapolated area was in normal cases approximately 10 per cent of the total area, and the accuracy of the extrapolation was increased by making double analyses of the two last samples of both the artery and the vein.

The cerebral metabolic rate of oxygen ($CMRO_2$) is calculated by

$$CMRO_2 = CBF \times (A - V)O_2 \quad \text{cc/100 g/min.} \quad \ldots\ldots \quad (7)$$

where $(A - V)O_2$ is the arterio-venous oxygen difference in volume per cent.

The cerebral vascular resistance (CVR) is calculated by

$$CVR = \frac{MABP - MJBP}{CBF} \text{ mm Hg/cc/100 g/min.} \quad \ldots\ldots \quad (8)$$

where MABP and MJBP are the mean blood pressures in mm Hg of the femoral artery and the internal jugular vein respectively.

Results.

The standard deviation of the individual determination of the cerebral blood flow. As described previously, the procedure for the determination of the cerebral blood flow was to take eight

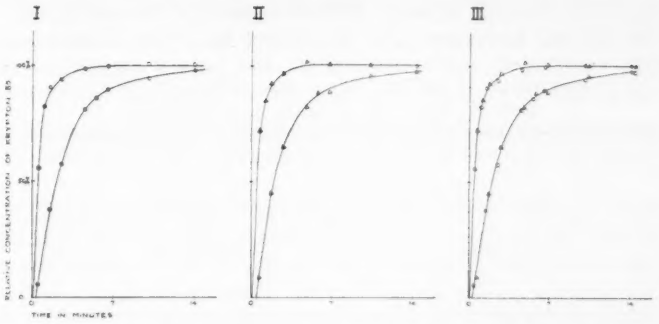

Fig. 6. The arterial and venous curves obtained in a duplicate determination of the cerebral blood flow. The procedure is described on page 37. Set I is drawn for the samples taken at the usual times, set II for the samples in the extra series. Set III is drawn for both sets of values.

Table 1.

Two left-side determinations of the cerebral blood flow made simultaneously on the same individual. 12 individuals were examined. (Procedure as described p. 37.)

	Cerebral blood flow cc/100 g/min.	
	I	II
	41.6	44.6
	46.8	46.2
	50.5	47.2
	45.4	47.6
	52.5	51.5
	55.3	53.5
	50.9	52.2
	59.0	57.4
	43.5	41.0
	37.2	37.8
	54.8	55.8
	77.3	73.2
Mean ...	51.2	50.6
S. D. ...	1.6	

$$\text{S. D.} = \sqrt{\frac{\Sigma\, D^2}{2n}}$$

samples from the artery and eight from the vein (fig. 6, set I). For the determination of the standard deviation of the cerebral blood flow each sample was duplicated, *i. e.* an additional sample

Table 2.

Two left-side determinations of the cerebral blood flow, arterio-venous oxygen difference, oxygen consumption, and vascular resistance made with an interval of 30 minutes on the same individual. 10 normal individuals, mean age 42 years, were examined.

	Cerebral							
	Blood flow cc/100 g/min.		Arterio-venous O_2 difference Vol. %		O_2 consumption cc/100 g/min.		Vascular resistance mm Hg/cc/100 g/min.	
	I	II	I	II	I	II	I	II
	43	40	7.0	7.0	3.0	2.8	2.4	2.9
	40	39	6.7	6.4	2.7	2.5	2.1	2.1
	51	48	4.5	4.9	2.3	2.4	1.9	1.7
	52	48	7.7	8.1	4.0	3.9	1.9	1.9
	51	50	6.2	6.5	3.2	3.3	1.5	1.5
	32	33	7.2	7.2	2.3	2.4	2.3	2.4
	59	63	6.7	6.0	4.0	3.8	1.7	1.5
	53	52	6.3	6.4	3.3	3.3	1.5	1.5
	39	43	9.7	8.8	3.8	3.8	2.3	2.1
	64	69	5.9	6.1	3.8	4.2	1.6	1.4
Mean	48.4	48.5	6.79	6.74	3.24	3.24	1.92	1.90
Mean diff.	0.1 $p > 0.05$		0.05 $p > 0.05$		0.0 $p > 0.05$		0.02 $p > 0.05$	
S. D. of the individual values for the cerebral oxygen consumption					0.13			

$$\text{S. D.} = \sqrt{\frac{\Sigma D^2}{2n}} \qquad t = \frac{\text{mean difference}}{\text{standard error of mean diff.}}$$

was taken immediately after each of the sixteen samples (fig. 6, set II).

The cerebral blood flow was calculated for each set, and the results of twelve such experiments are given in table 1. The standard deviation was 1.6 cc/100 g/min. for CBF values between 37 and 77 cc/100 g/min.

Successive measurements of the cerebral blood flow, oxygen consumption, and vascular resistance in the same individual. In ten normal subjects two determinations were made with an interval of thirty minutes. The experimental conditions were essentially the same during the two determinations with regard to posture, pulse rate, blood pressure, metabolic rate, hemoglobin percentage, and arterial carbon dioxide tension. The results are given in table 2.

The differences between the mean values of the cerebral functions studied were not significant. The individual changes in cerebral oxygen consumption were all very small and were not significantly correlated to the individual changes in the other functions studied. Regarding the experiments as duplicate determinations of cerebral oxygen consumption, the standard deviation of this function was 0.13 cc/100 g/min.

If calculated from the standard deviation of the cerebral blood flow and the standard deviation of the cerebral arterio-venous oxygen difference, 0.23 vol.%[1], the standard deviation of the cerebral oxygen consumption was 0.16 cc/100 g/min., a value not significantly different from that calculated from the results given in table 2.

In a similar study of a subject with slight hypertension a considerable difference between the values for the first and the second determination was found, as the cerebral blood flow increased 14 cc/100 g/min., the cerebral oxygen consumption increased 1.3 cc/100 g/min., and the cerebral vascular resistance decreased 1.1 mm Hg/cc/100 g/min. The discrepancy between the results referred to in table 2 and this observation is so great that we have not included this study when calculating the standard deviation of the cerebral oxygen consumption.

In 4 normal and 6 arteriosclerotic individuals, two determinations were made with an interval of three to eight days. The experimental conditions were not the same during the two determinations, as the mean arterial carbon dioxide tension was significantly higher in the second series, whereas the mean hemoglobin percentage and the mean arterial pressure were significantly lower. The results are given in table 3.

The mean cerebral blood flow increased significantly, whereas the mean arterio-venous oxygen difference and the cerebral vascular resistance decreased significantly. The mean cerebral oxygen consumption did not change significantly. In three of the nine individuals where repeated determinations of cerebral oxygen consumption were made, a significant variation of this function was noted. All three individuals were normal, and it can therefore be concluded that the cerebral oxygen consumption determined unilaterally may vary from day to day in normal subjects.

[1] The standard deviation of the cerebral arterio-venous oxygen difference was calculated from 12 duplicate determinations.

Table 3.

Two left-side determinations of the cerebral blood flow, arterio-venous oxygen difference, oxygen consumption, and vascular resistance made with an interval of three to eight days on the same individual. 4 normal (N) individuals and 6 arteriosclerotic (A) individuals were examined.

	Cerebral							
	Blood flow cc/100 g/min.		Arterio-venous O_2 difference Vol. %		O_2 consumption cc/100 g/min.		Vascular resistance mm Hg/cc/100 g/min.	
	I	II	I	II	I	II	I	II
A	33	40	6.3	4.6	2.1	1.9	2.5	2.0
A	19	24	8.5	—	1.6	—	5.7	—
N	50	47	7.4	6.5	3.7	3.1	2.1	2.0
A	33	41	5.6	4.4	1.8	1.8	3.2	2.4
A	29	33	5.3	4.3	1.5	1.4	3.6	2.7
N	49	51	7.3	6.9	3.4	3.5	2.0	1.6
N	62	64	6.6	4.7	4.1	3.0	1.9	1.4
N	48	67	5.3	5.0	2.5	3.4	2.5	1.3
A	25	28	6.2	7.0	1.6	2.0	5.7	4.3
A	29	28	8.1	7.3	2.3	2.0	5.0	4.2
Mean	37.7	42.3	6.45	5.64	2.56	2.46	3.17	2.44
Mean diff.	4.6 $p<0.05$		0.81 $p<0.02$		0.10 $p>0.05$		0.73 $p<0.001$	

$$t = \frac{\text{mean difference}}{\text{standard error of mean diff.}}$$

Normal Values.

In twenty healthy subjects, ten males and ten females, the cerebral blood flow, cerebral oxygen consumption, and the cerebral vascular resistance were determined. On the day of examination, the subjects were fasting and had been lying at rest for several hours. No premedication was used. Subjects with a metabolic rate higher than 135 % during the examination were not considered normal. Blood from the left internal jugular vein was used in all determinations. The essential data are given in detail in tables 4 and 5.

The cerebral functions studied could not be significantly correlated to sex, age, metabolic rate, arterial carbon dioxide tension, or mean arterial blood pressure.

The cerebral blood flow varied from 33 to 67 cc/100 g/min.,

Table 4.

Values for cerebral blood flow (CBF), cerebral arterio-venous oxygen difference ($C(A—V)O_2$), cerebral oxygen consumption ($CMRO_2$), and cerebral vascular resistance (CVR), in 20 normal subjects, 10 males and 10 females.

The age, weight, metabolic rate (MR), arterial carbon dioxide tension ($ApCO_2$), and mean arterial blood pressure (MABP), are given for each subject.

The cerebral venous blood was in all cases taken from the superior bulb of the left internal jugular vein.

	Age years	Weight kg	MR %	$ApCO_2$ mm Hg	MABP mm Hg	CBF cc/100 g/ min.	$C(A—V)O_2$ Vol. %	$CMRO_2$ cc/100 g/min.	CVR mm Hg/ cc/100 g/min.
MALES	17	66	108	33	95	51	7.2	3.7	1.7
	28	79	96	49	107	52	7.7	4.0	1.9
	30	72	98	49	74	62	6.1	3.8	1.1
	31	86	87	35	88	59	6.3	3.7	1.4
	35	68	120	31	106	39	8.1	3.2	2.5
	41	75	106	34	108	49	7.8	3.8	2.0
	43	87	85	30	100	51	5.9	3.0	1.8
	50	73	112	44	83	52	6.2	3.2	1.5
	54	76	133	42	106	52	7.0	3.6	1.9
	55	70	122	43	106	43	7.1	3.1	2.3
FEMALES	25	63	104	32	100	59	7.2	4.2	1.5
	28	59	118	33	91	53	8.2	4.3	1.6
	29	56	92	31	98	43	5.8	2.5	2.2
	29	75	87	31	87	33	7.2	2.4	2.4
	32	50	120	33	80	53	6.3	3.3	1.5
	41	56	120	41	107	67	4.4	2.9	1.5
	41	83	110	44	110	59	6.7	4.0	1.7
	45	65	124	38	112	63	5.9	3.7	1.7
	45	51	115	40	86	48	4.9	2.4	1.7
	54	48	108	38	108	43	7.0	3.0	2.4

the mean value was 52 cc/100 g/min., the standard deviation was 8.6 cc/100 g/min., and the standard error 1.9 cc/100 g/min.

The cerebral oxygen consumption varied from 2.4 to 4.3 cc/100 g/min., the mean value was 3.4 cc/100 g/min., the standard deviation was 0.58 cc/100 g/min., and the standard error was 0.13 cc/100 g/min.

The cerebral vascular resistance varied from 1.1 to 2.5 mm Hg/cc/100 g/min., the mean value was 1.8 mm Hg/cc/100 g/min., the standard deviation was 0.38 mm Hg/cc/100 g/min., and the standard error was 0.085 mm Hg/cc/100 g/min.

The cerebral functions studied are expressed per 100 g brain tissue, where the brain tissue is defined to include the blood in the cerebral vessels (cf. p. 35).

Table 5.

Mean values, ranges, standard deviations (S. D.), and standard errors (S. E.) of the cerebral blood flow, cerebral oxygen consumption, and the cerebral vascular resistance calculated from 20 left-side determinations in 20 normal subjects, 10 males (mean age 38 years) and 10 females (mean age 37 years). The individual results are given in table 4.

	Cerebral		
	Blood flow cc/100 g/min.	O_2 consumption cc/100 g/min.	Vascular resistance mm Hg/cc/100 g/min.
Mean	52	3.4	1.8
Range	33—67	2.4—4.3	1.1—2.3
S. D.	8.6	0.58	0.38
S. E.	1.9	0.13	0.085

$$\text{S. D.} = \sqrt{\frac{\Sigma\, D^2}{n-1}}$$

$$\text{S. E.} = \frac{\text{S. D.}}{\sqrt{n}}$$

The standard deviation of cerebral oxygen consumption of the normal material was significantly higher than the standard deviation of the individual determination of the cerebral oxygen consumption, indicating that cerebral oxygen consumption, calculated from unilateral determinations, is not constant from one normal individual to another.

Discussion of the Method.

In the following, the Kr^{85} method for the determination of cerebral blood flow will be compared to the nitrous oxide method developed by KETY and SCHMIDT (1945, 1948a).

Using 6 mC Kr^{85} in a closed system of 60 liters, the admixture of foreign inert gas is of the order of 2×10^{-4} per cent, a concentration which can be considered not to influence the functions studied. This cannot be stated a priori with the same degree of safety regarding the inhalation of 15 per cent nitrous oxide used by KETY and SCHMIDT, even though no appreciable physiological or mental effects were noted by those authors. In this connection it is of considerable interest that the normal values of the two methods are not significantly different.

The metabolic rate during the examination can easily be calculated when using the Kr^{85} method as described, whereas it is more difficult when using the ordinary nitrous oxide method. For the study of the influence of various gas mixtures, the nitrous oxide method is superior.

Measurement of the relative concentration of Kr^{85} in blood samples with the technique described is specific, accurate, and rapid. Volumetric nitrous oxide analysis does not differentiate between nitrous oxide and nitrogen, and must therefore be considered less specific. The reproducibility of the two analyses is comparable, as the standard deviation of volumetric nitrous oxide analysis was found to be 1 to 2 per cent (KETY and SCHMIDT 1948a). Each Kr^{85} analysis requires about two minutes of manual work, whereas about fifteen minutes are necessary for each volumetric nitrous oxide analysis.

A problem specific for the Kr^{85} method is the exposure of the examiners and the subjects to ionizing radiation. Owing to the exclusive emission of weak beta-rays and to the carrier capacity of atmospheric air, the radiation exposure of the examiners is quite negligible. The radiation doses to which the subjects are exposed during the examination have been calculated (see supplement II) and were found to be quite small, especially when allowance was made for the exceptionally short time of exposure.

The calculation of cerebral blood flow from the graphs is based on the Fick principle, which was also used by KETY and SCHMIDT (1945, 1948a); however the calculation used in this paper is more elaborate, as four additional factors have been taken into account: 1) the mean circulation time lung—brain, 2) the mean circulation time through the brain, 3) a complete equilibrium between brain and blood in each case at the time when the concentration in the brain is calculated from the blood concentration and the partition coefficient brain:blood, and 4) the variations of the partition coefficient brain: blood with the hematocrit value. The value for the cerebral blood flow of normal individuals is not affected materially by the more elaborate calculation, but in pathological cases the four factors mentioned may exert considerable influence.

Discussion of the Results.

As the standard deviation of the cerebral blood flow, measured by use of nitrous oxide has not been determined experimentally, the accuracy of the two methods cannot be compared directly. But, as the individual blood samples are analysed with approximately the same accuracy, the Kr^{85} method is considered more exact, as each set of graphs is determined by 20 analyses when using the Kr^{85} method and only by 10 when the original nitrous oxide method is applied.

From the standard deviations for cerebral blood flow and for cerebral arterio-venous oxygen difference, the standard deviation

of cerebral oxygen consumption was calculated to be 0.16 cc/100 g/min. Repeated determinations of cerebral oxygen consumption at an interval of 30 minutes in ten subjects showed only variations within the calculated margin of error of the method. In one subject, however, a highly significant change in cerebral oxygen consumption was observed. It is assumed that this variation is of the same nature as the variations which may be observed in the same individual when examined on different days.

Repeated determinations using the original nitrous oxide method, made at an hour's interval in normal subjects, have been reported by LAMBERTSEN et al. (1953), who found no significant changes in any of the functions studied. As only the mean values were published, it is not possible to compare the standard deviation for cerebral oxygen consumption in their series to the value of 0.13 cc/100 g/min., obtained in our series. Repeated determinations, using the original nitrous oxide method made at an interval of 20 minutes in schizophrenic patients given small amounts of amobarbithal and thiopenthal during the second determination, have been reported by KETY, WOODFORD, HARMEL, FREYHAN, APPEL, and SCHMIDT 1948. They found no significant changes in the mean values, and the standard deviation of cerebral oxygen consumption was calculated to be 0.19 cc/100 g/min., a value not significantly higher than the value obtained in our series. A significantly higher standard deviation for cerebral oxygen consumption of 0.73 cc/100 g/min. can be calculated from the results of HAFKENSCHIEL and FRIEDLAND (1953), who made repeated determinations, at an interval of 60 minutes, using the original nitrous oxide method, in 6 hypertensive subjects and 6 subtotally adrenalectomised hypertensive subjects, all given a placebo injection during the second determination.

The cerebral metabolism was restudied in the same subject at an interval of a few days (table 3), and significant variations in cerebral oxygen consumption were noted in some of the individuals. This is in agreement with the results reported by KETY and SCHMIDT (1946). The observed variations in mean cerebral blood flow, cerebral arterio-venous oxygen difference, and cerebral vascular resistance may be attributed to the changes in hemoglobin percentage (cf. HEYMAN et al. 1952, and SCHEINBERG 1951), or to changes in respiration as evidenced by the altered arterial carbon dioxide tension (cf. KETY and SCHMIDT 1946, 1948b).

Table 6.

Mean values and standard deviations of the cerebral blood flow, cerebral oxygen consumption, and cerebral vascular resistance in normal subjects as reported in the literature.

	Kety and Schmidt (1948)		Scheinberg and Stead (1949)		Bernsmeier and Siemons (1953)		Lassen and Munck (1955)	
	Mean	S. D.	Mean	S. D.	Mean	S. D.	Mean	S. D.
Cerebral blood flow (cc/100 g min.)..	54	12	64.7	12.1	58.3	6.6	52	8.6
Cerebral oxygen consumption (cc O_2/100 g/min.)..	3.3	0.4	3.8	0.6	3.7	0.4	3.4	0.6
Cerebral vascular resistance (mm Hg/cc/100 g/min.)	1.6	0.4	1.3	0.2	1.5	0.3	1.8	0.4

The normal values given in tables 4 and 5 do not differ significantly from the values reported by Kety and Schmidt (1948a). Application of the Kety—Schmidt formula (Kety and Schmidt 1948a) for S = 1.10, on the observations made using Kr^{85}, does not alter the normal values significantly. Scheinberg and Stead (1949) and Bernsmeier and Siemons (1953), who used a modification of the nitrous oxide method (Scheinberg and Stead 1949), reported normal values which are significantly different from the results presented here (table 6).

Similar to our results, the normal series of Kety and Schmidt (1948 a), of Scheinberg and Stead (1949), and of Bernsmeier and Siemons (1953) showed considerable inter-individual variations in cerebral oxygen consumption. It cannot be excluded that these inter-individual variations are an effect of those which may occur from day to day in the same individual, or that the two types of variation have a common cause.

Summary.

1) A method for the determination of cerebral blood flow in man using the radioactive isotope krypton 85 is described.

2) The standard deviation of cerebral blood flow was experimentally determined to be 1.6 cc/100 g/min.

3) Repeated determinations of cerebral oxygen consumption at an interval of 30 minutes showed insignificant variations in 10 normal subjects and significant variations in 1 subject with slight hypertension.

4) Repeated determinations of cerebral oxygen consumption at an interval of 3 to 8 days showed significant variations in 3 out of 4 normal individuals and in none out of 5 arteriosclerotic individuals.

5) In a group of 10 normal males and 10 normal females, mean age 38 years, the mean value for cerebral blood flow was 52 (S. D. = 8.6) cc/100 g/min., the mean value for cerebral oxygen consumption was 3.4 (S. D. = 0.58) cc/100 g/min., and the mean value for cerebral vascular resistance was 1.8 (S. D. = 0.38) mm Hg/cc/100 g/min.

6) The method described is compared to the nitrous oxide method as described by KETY and SCHMIDT. The results of the two methods agree, but the krypton 85 method is more accurate, secures more normal physiological conditions during the examination, and demands less manual work.

Supplement I.

THE PARTITION COEFFICIENT BRAIN : BLOOD (S).

The partition coefficient S is defined by:

$$S = \frac{C_{brain}}{C_{blood}} \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

where C_{brain} = units Kr^{85} per g brain tissue, and
C_{blood} = units Kr^{85} per cc blood
at the same tension of Kr^{85} in brain tissue and blood, and at body temperature. The brain tissue is defined as including the blood in the cerebral vessels. C_{blood} can be expressed:

$$C_{blood} = F_1 \times C_{rbc} + F_2 \times C_{pl} \quad \ldots\ldots\ldots\ldots \quad (2)$$

where C_{rbc} and C_{pl} are the concentrations of Kr^{85} in the red blood corpuscles and in plasma respectively, and F_1 and F_2 are ratios expressing the fractions of red blood corpuscles (F_1) and plasma (F_2) in 1 cc blood, *i. e.* $F_1 + F_2 = 1$, and $F_1 \times 100 =$ the hematocrit value.

As C_{pl}/C_{rbc} was determined experimentally and was found to be 0.685 (S. E. = 0.005), it follows from (1) and (2) that S varies with the hematocrit value. By appropriate substitution (2) is changed to:

$$S = \frac{2 \times C_{brain}}{C_{rbc} + C_{pl}} \times \frac{0.843}{F_1 + 0.685 \times F_2} = S' \times \frac{0.843}{F_1 + 0.685 \times F_2} \quad \ldots \quad (3)$$

Table 7.

The results of four animal experiments performed in order to determine the partition coefficient brain:blood for Kr^{85} at 37° C.

		Exp. I	Exp. II	Exp. III	Exp. IV
Recorded number of emissions (background value subtracted)	Brain	7,540	8,213	7,383	6,417
	Blood	6,612	7,447	6,602	5,674
	Water	7,494	8,099	7,360	6,351
Specific gravity of	Red blood corpuscles	1.080	1.080	1.086	1.082
	Plasma	1.029	1.033	1.030	1.031
S'	Individual value ..	1.077	1.040	1.052	1.066
	Mean value	1.059			
	Standard error	0.0075			

S' was calculated according to formula (4).

For the experimental determination of S', one cuvette of brain tissue was compared with one cuvette of blood. The relative contents of Kr^{85} in layers of equal weight are compared by this procedure. As C_{blood} is defined as units Kr^{85} per cc, S' can be expressed:

$$S' = \frac{\text{emissions/min./cuvette brain tissue}}{\text{emissions/min./cuvette blood} \times G} \quad \ldots\ldots\ldots \quad (4)$$

where $G = \frac{G_{pl} + G_{pl} \times 0.685}{G_{pl} + G_{rbc} \times 0.685} \times G_{rbc}$, G_{pl} and G_{rbc} are the specific gravities of plasma and red blood corpuscles respectively. Correction for the specific gravities has also been used when determining C_{pl}/C_{rbc}.

The experimental procedure for the determination of S' was as follows:

The experiment was performed at 37° C. An anaesthetised cat was placed in an air-tight box containing approximately 20 liters of atmospheric air with about 2 mC Kr^{85}, and a water bath of 1 liter which was stirred continually. The necessary manipulations could be made through two holes fitted with cuffs. After 60 minutes, anaerobic samples were taken of water and arterial blood. Immediately thereafter the cat was decapitated. The brain was then removed and submerged in the bath. As described by KETY, HARMEL, BROOMELL, and RHODE (1948), a 5 cc syringe was filled with brain tissue and homogenised by means of a piece of steel wire inserted through the nozzle.

The syringe was then sealed by fixing a cuvette filled with water from the bath to the nozzle of the syringe. The syringe and cuvette were removed from the water, and the brain tissue expelled from the syringe into the cuvette, which was then sealed. The blood and water samples were transferred to cuvettes. The specific gravities of plasma and red blood corpuscles were determined by the $CuSO_4$ method.

The results of four experiments are given in table 7.

The mean value of S' was found to be 1.06, and S was then calculated for different hematocrit values according to formula (3).

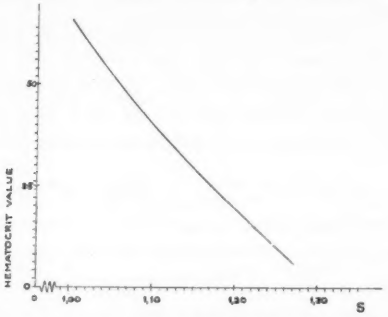

Fig. 7. The variation of the partition coefficient brain:blood, S with the hematocrit value.

Table 8.

The radiation dose in r. e. p. (roentgen equivalent physical, corresponding to an energy absorption of 93 erg per g tissue), and the radiation dose rates in r. e. p./min. calculated for an organism weighing 70 kg, in equilibrium for 14 minutes with an atmosphere containing 0.1 mC Kr^{85} per liter. The total amount of ionizing radiation absorbed by the whole organism would, when absorbed in air, deliver 140 roentgen.

		Radiation dose in r. e. p.	Radiation dose rate in r. e. p. per min.
Exposure to Kr^{85} 0.1 mC/liter air for 14 minutes	Trachea, superficial 0.05 mm	1.6×10^{-1}	1.1×10^{-2}
	Lungs	4.0×10^{-2}	2.9×10^{-3}
	Trachea	3.0×10^{-2}	2.2×10^{-3}
Exposure to Kr^{85} 0.005 mC/kg tissue[1] for 14 minutes	Total organism .	8.1×10^{-4}	5.4×10^{-5}

[1] The partition coefficient air:tissue for Kr^{85} is approximatively 20 : 1 (LAWRENCE et al. 1946).

Supplement II.

The radiation doses and radiation dose rates absorbed by the subject during a determination of the cerebral blood flow by the Kr^{85} method, are given in table 8.

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Observations on the Water and Electrolyte Metabolism in the Goat.

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The extensive studies by DENTON et al. (1951 and 1952) on excess sodium subtraction in the sheep give much valuable information on the electrolyte metabolism in a ruminant animal. But nevertheless it must be said that our knowledge of the special features in the normal metabolism of water and of electrolytes in these animals is very incomplete.

The polydipsic effect of intrahypothalamic injections of hypertonic NaCl-solutions in the goat has been described (ANDERSSON 1953). It was there observed that even animals, which had not drunk any water after receiving the injections sometimes developed a marked polyuria during the experiments. This polyuria was also now and then seen in animals during the preparations for an experiment before any incision had been made in the brain.

In order, if possible, to gain a clearer knowledge as to the causes of this polyuria and better information on the normal water and electrolyte metabolism in goats the experiments reported in this paper were undertaken.

Methods.

For the experiments 6 female, fullgrown and not lactating goats were used. The animals were fed on hay, some crushed oats and fragments of hard bread, and were always offered water ad libitum. Three

of the animals (Group 1) had free access to NaCl in the form of block salt, whereas the other three goats had not had any extra salt for more than three months (Group 2). Four of the animals had been provided with closed ruminal fistulae some weeks before the experiments started.

An experiment was as a rule started at about 10 o'clock a. m., when the urinary bladder was emptied by catheterisation and a blood sample was taken from the jugular vein. The animal was then taken from the stable to the laboratory where it was placed in a modified Pavlov stand and where its diuresis was recorded continuously by help of a "Dowse's" selfretaining catheter. The urine was mostly collected in 10 minutes' samples, and between the samples the collecting system was washed with 10 cc of distilled water. Blood samples were taken during the experiments from the left jugular vein, and intravenous injections and infusions were made into the right jugular vein.

Where the glomerular filtration rate was measured by inulin clearance a priming dose of 1.5 g of inulin in a 10 % solution was given, followed by a constant intravenous infusion of 3.5 g of inulin per hour. The first clearance period started about 40 minutes after the priming dose of inulin.

Inulin was determined according to JOSEPHSON and GODIN'S (1943) modification of CORCORAN and PAGE'S (1939) method.

The hematocrit values were determined by centrifugation at 3,000 r. p. m. for 30 minutes, using heparinized blood in Wintrobe tubes.

Sodium and potassium analyses in plasma and urine were made, using the EEL flame photometer.

Plasma chloride determinations were made according to the silver nitrate method of VAN SLYKE, and urine chloride according to the Volhard method (PETERS and VAN SLYKE 1932).

The experiments can be classified under four headings:

A) A study of the response of the animals to big doses of water, administered through ruminal fistula.

B) Inhibitory effect of posterior pituitary extract on the water diuresis in these animals and its influence on the excretion of certain electrolytes by the kidney.

C) Changes in the rate of urine secretion and extracellular electrolyte pattern during certain emotional stress.

D) The effect of adrenaline and ephedrine on the diuresis and the urinary excretion of certain electrolytes.

In the sequel the following symbols will be frequently used:

GFR = Glomerular filtration rate.

P_{Na}, P_K and P_{Cl} = Plasma concentration of sodium, potassium and chloride.

U_{Na}, U_K and U_{Cl} = Urinary concentration of sodium, potassium and chloride.

Results.

A) Hydration Experiments.

The rate of urine secretion, the urinary excretion of Na^+, K^+ and of Cl^-, and changes in the blood composition, were studied after administering various amounts of bodywarm water into the rumen of four goats supplied with closed ruminal fistulae. Two of the animals (nos. 1 and 2) belonged to Group 1 (free access to NaCl) and the other two (nos. 3 and 4) belonged to Group 2 (no extra salt). The body weights of these four animals were within the range of 35—40 kg.

It was found that rather big doses of water could be introduced into the rumen at one time without causing any obvious discomfort to the animals. Thus water up to 20 % of their body weight was given in several experiments, and the subsequent water diuresis was recorded for different lengths of time. Fig. 1 shows a graph of such an experiment in animal no. 3 where the complete course of the water diuresis, after 5,500 cc of water had been introduced at one time into the rumen, was followed. The diuresis here went up from 50 cc/hour to about 400 cc/hour within one hour after the water was given, and it remained fairly constant at this level for 6 hours when it started to decline gradually and reached prehydration level first after more than 13 hours.

When larger amounts of water (7—8,000 cc) were given the maximal diuresis measured sometimes reached 1,200 cc/hour. The course of the diuresis however was similar to that seen in Fig. 1. The highest rates of urine secretion in the hydration experiments were seen when the animals showed signs of unrest and irritation during the experiments.

If the goats were fed for a 10 minutes' period or longer during the course of the water diuresis this was often found to cause an inhibition of the polyuria of various degrees, lasting for 30 minutes to one hour.

P_{Na} showed only small changes during these experiments. From a prehydration level of 145 to 150 m Eq/l in both groups of animals a small fall was observed during the first hour or two of hydration, but then it rose again to approximately the prehydration level. A fall of P_{Cl} could however be observed from a prehydration level of about 105 m Eq/l to 95—100 m Eq/l

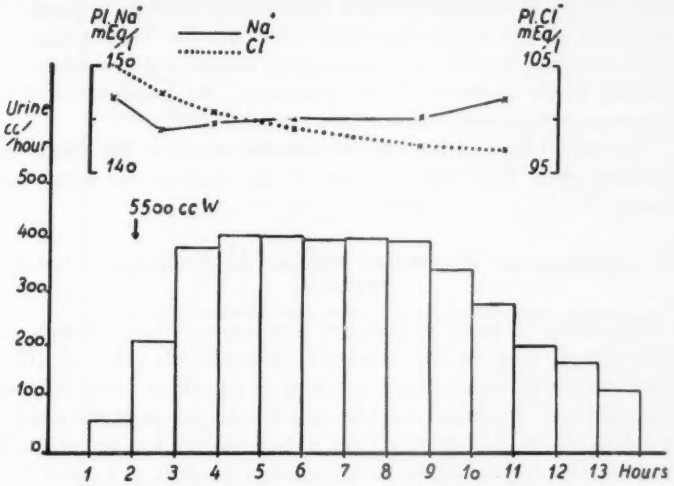

Fig. 1. The course of the water diuresis and changes of P_{Na} and P_{Cl} in goat no. 3 after the administration of 5,500 cc of body warm water in one dose into the rumen. The body weight of this animal before the administration of water was 37 kg.

four to five hours after the hydration started. Similarly the urinary excretion of Na^+ remained fairly constant in the animals belonging to Group 1 during the experiments, whereas the excretion of Cl^- gradually declined.

There was, however, in these and the experiments reported in the sequel a marked difference in the rate of urinary excretion of Na^+ and Cl^- between the animals which had had free access to NaCl and those which had had no extra salt. Thus the goats belonging to Group 1 in some experiments excreted more than 1 m Eq/min. of Na^+ and of Cl^-, whereas the excretion of these electrolytes in animals of Group 2 was minimal especially during the first part of an experiment. On most occasions the urine collected when emptying the bladder before an experiment in these latter animals was found to contain only traces of Na^+ when the K^+ concentration was as high as 200—300 m Eq/l. Thus U_K/U_{Na} ratios as high as 300 were found in concentrated urine of the Group 2 animals.

P_K was generally within the range of 4—5 m Eq/l in both groups of animals and the excretion of K^+ remained fairly con-

stant during the course of most experiments, though in some it decreased slowly as the experiment went on. The highest U_K/P_K ratio observed was 70 (in concentrated urine) and the lowest 2 (during water diuresis). Where measured, the potassium/inulin clearance ratios were within the range 0.5—0.7.

The initial hemoglobin and hematocrit values of the Group 2 animals were higher than those of the animals belonging to Group 1.

B) Inhibition of the Water Diuresis by Posterior Pituitary Extract.

The effect of small amounts of posterior pituitary extract on the diuresis and on the urinary excretion of Na^+, K^+ and Cl^- was studied in the animals hydrated as described above. 5—10 milliunits of "Pituitrin" (PARKE and DAVIS) given intravenously resulted in an inhibition of the water diuresis lasting from 30 minutes to one hour. In both groups of goats U_{Na}, U_K and U_{Cl} rose during this inhibition, but whereas in animals of Group 1 the urinary concentration of Na^+ and Cl^- rose well above the plasma levels this was far from being the case in the animals of Group 2. In these latter animals the rate of urinary excretion of Na^+, K^+ and Cl^- did not change during the inhibition of the water diuresis, but an increase of up to 30 % in the rate of excretion of Na^+ and Cl^- was observed during the inhibition in the animals fed with free access to NaCl. No such increase was seen in the excretion of K^+.

Fig. 2 shows such an experiment in animal no. 2, belonging to the saltfed group.

C) Changes in the Rate of Urine Secretion and Extracellular Electrolyte Pattern during Certain Emotional Stress.

In experiments on goats, where the urine secretion was followed under various conditions, a certain instability in their diuresis was noticeable. Thus it appeared that animals, especially when restless and irritated, after having been catheterized and placed in the Pavlov stand with no access to food, within 30 minutes to one hour developed a marked polyuria. The specific gravity of the urine in these cases fell from between 1.015—1.030 in the urine samples first collected to 1.001—1.005, and the rate of urine secretion sometimes increased 10-fold. This polyuria could

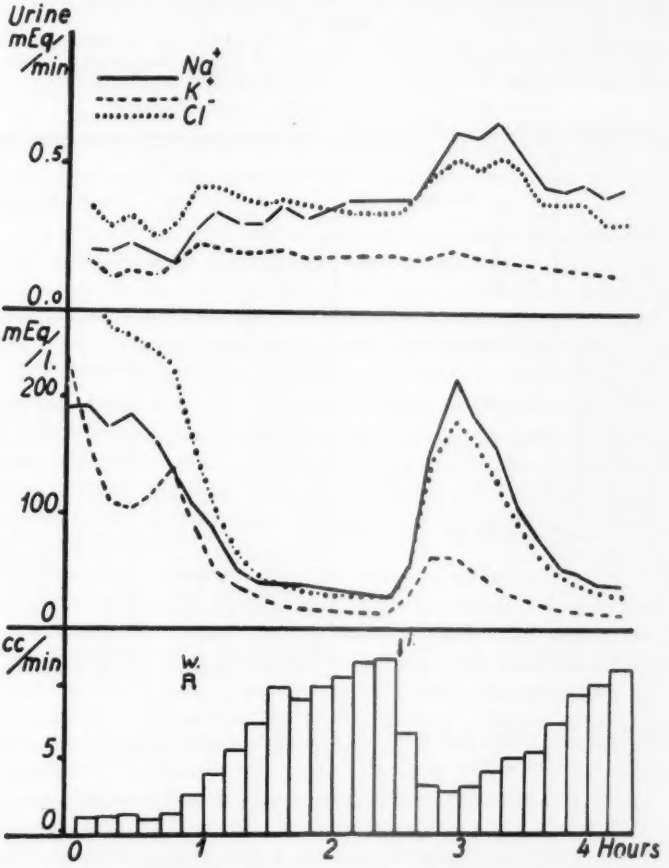

Fig. 2. The effect of "Pituitrin" on the water diuresis and the urinary excretion of Na, K and Cl in animal no. 2 (belonging to the saltfed group).
W: 6,000 cc of body warm water administered into the rumen through ruminal fistula.
i.: The intravenous injection of 10 milliunits of "Pituitrin".

last for 2—3 hours, but it was generally inhibited within 15—30 minutes if the animals were fed and ate heartily. In the same manner the polyuria disappeared if the animals started to ruminate and continued to do so for some time. The polyuria resembled a water diuresis in the further respect that it was in-

Table 1.

The protocol of an experiment in goat no. 6 (fed without access to extra salt) showing typical changes in plasma electrolyte pattern and rate of urine secretion during certain emotional stress.

Time	Blood					Urine								
			Plasma m Eq/l					m Eq/l			m Eq/10 min.		
	g Hb/100	Hematocrit	Na^+	K^+	Cl^-	Vol. cc/10 min.	Spec. gr.	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-
10.45	13	42	146.5	4.6	104		1.015	trace	192	51			
10.50—55						32	1.010	1.5	97.5	32	0.05	3.1	0.8
11.00—10						38	1.005	1.0	62.5	16			
10—20	12	38	148	4.3	103.5	53	1.003	1.2	55	12	0.06	2.9	0.6
20—30						66	1.002	1.2	44	9	0.08	2.9	0.6
30—40	11.5	36	149	4.5	103	77	1.001	—	—	8	—	—	0.6
40—50						76	»	1.6	34	7	0.1	2.6	0.6
50—00						85	»	—	—	8	—	—	0.7
12.00—10						83	»	2.1	30	8	0.2	2.5	0.7
10—20						74	»	4.0	37	9	0.3	2.7	0.7
20—30	12	37	151	4.0	102.5	69	»	9.0	42	12	0.6	2.9	0.8
30—40						54	1.003	21.0	55	16	1.2	3.0	0.9
40—50						36	1.004	37.0	74	27	1.3	2.7	1.0
50—00						32	1.007	52.0	86	30	1.6	2.7	0.9
13.00—10						26	1.010	65.0	94	33	1.7	2.4	0.8
10—20	11	35	151	3.85	102.5	27	1.009	75.0	87	31	2.0	2.4	0.8
20—30						32	1.008	78.0	78	28	2.5	2.5	0.9
30—40						33	1.007	73.0	66	25	2.4	2.2	0.8

hibited for about one hour by the intravenous injection of small amounts of posterior pituitary extract (5 milliunits of "Pituitrin").

This polyuria was most often observed and was of greatest intensity in the animals of Group 2. There P_{Na} rose somewhat as the polyuria developed whereas a fall of P_{Cl} was seen. The hemoglobin and hematocrit values were often lowered during the period of polyuria. In these animals, whilst the urinary excretion of Na^+ was minimal when the experiment started and during the first part of the polyuria, there was a marked rise in U_{Na} and the urinary excretion of Na^+ when the polyuria started to decline. There was however no similar rise in U_{Cl} and the excretion of Cl^-.

Table 1 shows the figures of an experiment of this kind in goat no. 6, which had been fed without access to extra salt for more than three months.

D) The Effect of Adrenaline and Ephedrine on the Diuresis and the Urinary Excretion of Na^+, K^+ and Cl^-.

As polyuria often developed during mild emotional stress in the goats it seemed to be of interest to study the effect of adrenaline and ephedrine on the secretion of urine of the animals.

It appeared that 0.5 mg of adrenaline or 5 cg of ephedrine given intravenously in most cases caused an intense polyuria. In such experiments, however, the state of hydration of the animals seemed to be of importance. For in experiments where the basal diuresis was very low and the urine of high specific gravity (more than 1.030) the polyuric response to adrenaline and ephedrine was sometimes nil, and even when positive was inconstant. But in cases where the basal diuresis was not markedly low the intravenous injection of 0.5 mg of adrenaline increased the rate of urine secretion up to 4 times the preinjection level for 5 to 10 minutes after the injection. Then it rapidly returned to the preinjection level.

The polyuria observed after the intravenous injection of 5 cg of ephedrine was of considerably longer duration (30 minutes to one hour). It did not develop as suddenly as that seen after the injection of adrenaline and it declined more slowly. At the height of the diuresis it was however of the same magnitude as that seen after adrenaline. The specific gravity of the urine during adrenaline and ephedrine polyuria fell to low values (1.001—1.005).

In animals hydrated through ruminal fistula, where the water diuresis remained at a level of about 10 cc/min. the intravenous injection of adrenaline and ephedrine increased the diuresis temporarily up to 20 cc/min., *i. e.* to nearly the maximal rate of urine flow ever seen in the same animal when strongly hydrated. But under conditions where the water diuresis was probably almost maximal (about 20 cc/min.), the intravenous injection of adrenaline and ephedrine did not cause any further increase in the rate of urine flow. If, on the other hand, such a "maximal" water diuresis was inhibited by the intravenous injection of 10 milliunits of "Pituitrin", the polyuric effect of adrenaline and ephedrine appeared during the period of inhibition. Under such conditions the injection of adrenaline and ephedrine could for

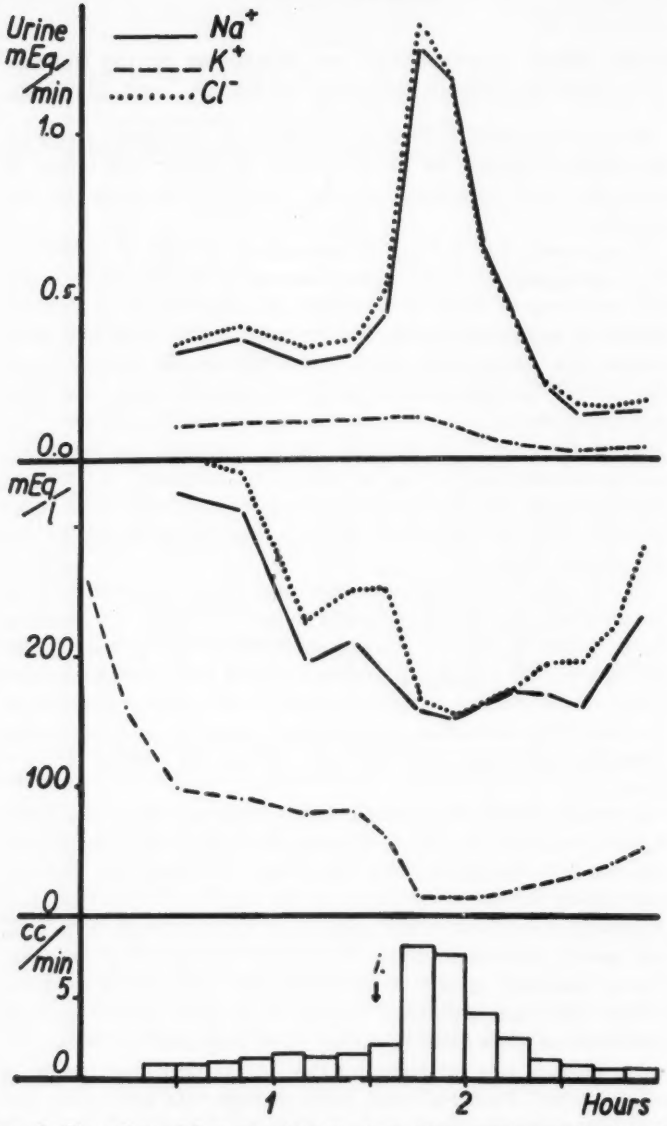

Fig. 3. The effect of 5 cg of ephedrine, given intravenously, on the diuresis and the urinary excretion of Na, K and Cl in goat no. 2 (fed with free access to NaCl).

i.: The intravenous injection of 5 cg of ephedrine.

a short while increase the rate of urine flow to almost the level it had been before the injection of "Pituitrin".

The intravenous injection of 5 cg of ephedrine into the goats caused an increase in hemoglobin and hematocrit values of up to 20 %. When such an injection was made into animal no. 6 (body weight 36 kg) in an experiment where the contemporary inulin clearance was studied, this procedure caused an increase in GFR from about 80 cc/min. before the injection of ephedrine to 95—90 cc/min. during three 10 minutes' periods following the injection. Ephedrine was not found to cause any marked change in P_{Na}, P_K or P_{Cl} in either group of goats. In both, however, there was a considerable increase in the *urinary excretion* of Na^+ and Cl^- during the adrenaline and ephedrine polyuria. Thus the intravenous injection of 5 cg of ephedrine could, in the animals fed with free access to NaCl, cause an increase in the excretion of Na^+ and of Cl^- from about 0.3 m Eq/min. before the injection to more than 1 m Eq/min. during two following 10 minutes' periods. Similarly the urinary excretion of Na^+ and of Cl^- in animals of Group 2 (no extra salt) increased from minimal amounts to about 0.5 m Eq/min. as a consequence to the injection of ephedrine.

Between the two groups there was however a marked difference in the *urinary concentration* of Na^+ and of Cl^- before and during the ephedrine polyuria. In animals fed with free access to NaCl, where U_{Na} and U_{Cl} were high before the injection of ephedrine, they fell towards the plasma levels. But in the other group of animals, where U_{Cl} and especially U_{Na} were extremely low before ephedrine was injected, this injection, in spite of consequent increased rate of urine flow, caused a considerable rise of U_{Na} and U_{Cl} during half an hour following the injection of ephedrine.

The *excretion of* K^+, however, in both groups of animals behaved differently to that of Na^+ and Cl^-. Thus only a small increase in the excretion of K^+ could be observed as a consequence of the intravenous injection of 5 cg of ephedrine.

Fig. 3 shows the effect of 5 cg of ephedrine given intravenously on the diuresis and the urinary excretion of Na^+, K^+ and Cl^- in animal no. 2, belonging to Group 1 (access to NaCl), and Fig. 4 a similar experiment in animal no. 6, belonging to Group 2 (no extra salt).

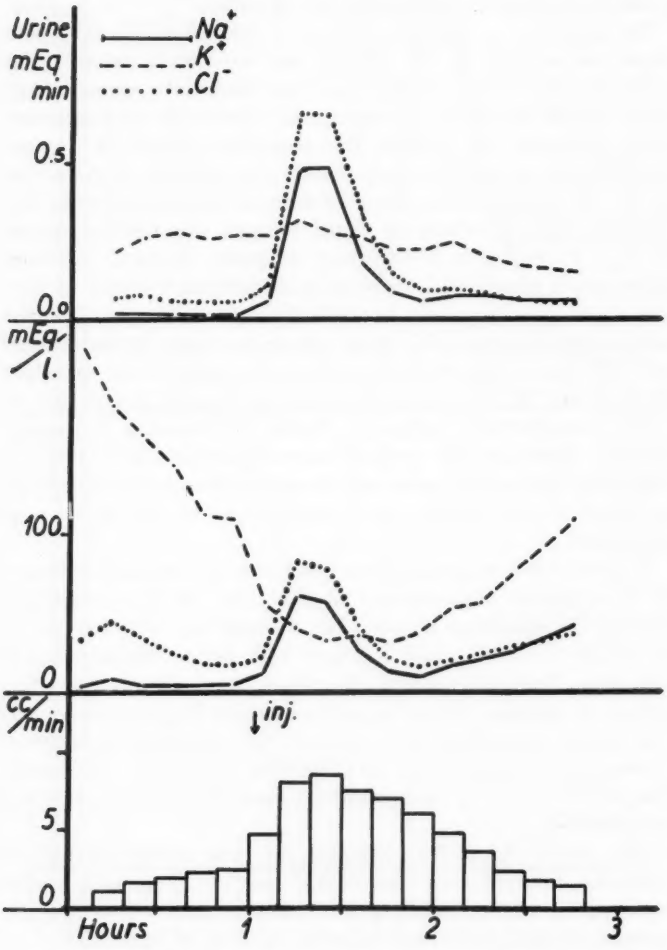

Fig. 4. The effect of 5 cg of ephedrine, given intravenously, on the diuresis and the urinary excretion of Na, K and Cl in goat no. 6 (no access to extra salt). inj.: The intravenous injection of 5 cg of ephedrine.

Discussion.

The anatomy of the alimentary canal in ruminants, differing as it does greatly from that of nonruminants, provides the conditions for special peculiarities in the water and electrolyte metabolism of the former animals. The rumen, which provides an important reservoir for food and liquid, and the copious and more or less continuous secretion of saliva (ECKHARD 1869, BABKIN 1949) in these animals, plays the major part in this. Thus DENTON et al. (1951, 1952) in their works on experimental sodium subtraction in the sheep have pointed out that the Na^+ concentration of the saliva in these animals approximately equals the extracellular Na^+ concentration, and that the Cl^- concentration is only about 20 % of the extracellular Cl^- concentration, whereas HCO_3^- in the saliva is concentrated 3—5 times more than in the extracellular fluid. The extracellular fluid in ruminating animals must therefore, according to DENTON et al., be submitted to considerably greater stresses as a consequence of the digestive processes than in most other species. This should chiefly depend on the fact that variations in the flow of saliva can cause great variations in the ruminal content of saliva and thus of electrolytes.

The hydration experiments described above show that the rumen also can be of importance as a reservoir of water. The fact that as much water as 20 % of the body weight could be put at one time into the rumen without causing any signs of water intoxication suffices to show that the absorption of water swallowed into the rumen must be a much slower process than the absorption of water in animals with a single stomach. The experiments do not show to what degree the absorption of water takes place through the ruminal wall, but it is reasonable to suggest that a great part of the water is absorbed only after further transport to the omasum and abomasum. The course of the diuresis, however, shows that if there is sufficient surplus of water in the rumen, there is a fairly constant absorption of water for several hours after its administration.

The fall of P_{Cl} as compared with the relatively unchanged P_{Na} during the course of the hydration can probably be explained as follows. When the animal has to go without food and consequently the secretion of saliva decreases, the continual reabsorp-

tion of Na^+ and HCO_3 in excess of Cl^- from the rumen causes a fall of the extracellular Cl^-/Na^+ ratio. This in its turn causes a gradual fall in U_{Cl}/U_{Na} ratio.

The difference in urinary excretion of Na^+ and Cl^- in the animals belonging to Group 1 (free access to NaCl) and those of Group 2 (no extra salt) shows that the goat kidney behaves as that of other species when there is simple electrolyte deprivation. The excretion of Na^+ could thus be practically nil in the Group 2 animals during the first part of an experiment. The higher hemoglobin and hematocrit values found in these latter animals might be a sign of a reduced blood volume with subsequent lower GFR in comparison with that of the animals belonging to Group 1. As a reduced GFR would mean a decreased load of electrolytes delivered to the tubular reabsorptive system, this might explain why the reabsorption of Na^+ and Cl^- here was almost complete, although the plasma concentrations of these electrolytes were normal. To get an answer to this question a comparison between the GFR of the two groups of animals would have to be made, combined with adequate blood and extracellular volume determinations. Another possible explanation is that dietary restriction has in some manner caused increased elaboration of Na+ retaining adrenal corticoids as suggested by BLACK et al. (1950) and by FRIEDEN et al. (1952).

There is much evidence to show that posterior pituitary extract has no effect on the urinary excretion of Na^+ and Cl^-, but on the other hand many investigators, mostly using large doses of posterior pituitary extract, have found it to increase the urinary excretion of these electrolytes. The dose of "Pituitrin" (10 milliunits) given here was just sufficient to give a moderate inhibition of the water diuresis in the goats. This dose did not change the urinary excretion of Na^+, K^+ and Cl^- in the animals on a low dietary intake of NaCl. In those fed with free access to NaCl it caused a small increase in the rate of excretion of Na^+ and Cl^- during the period of inhibition. MUELLER et al. (1951) have suggested that changes in GFR so slight as not to be detectable by present methods may account for modifications in the urinary excretion of sodium. A small increase in GFR due to some increase of extracellular volume during pituitary inhibition of the water diuresis might well have caused the small increase in Na^+ and Cl^- excretion observed in the Group 1 animals. The experiments thus provide no real evidence for any

natriuretic or chloruretic effect of posterior pituitary extract in the goat.

From the results no conclusions can be drawn as to the nature of the polyuria seen during certain emotional stresses in the goats. The changes observed here in extracellular electrolyte pattern are similar to those observed in sheep within four to eight hours of nonspecific trauma (WHITE 1951; HARRIS, MCDONALD and WILLIAMS 1952). An inhibition of the salivary secretion, due to emotion, and a continued absorption of Na^+ in excess of Cl^- from the alimentary canal, might, especially in the saltdepleted animals lead to an expansion of the extracellular fluid. This, especially if combined with increased arterial blood pressure, might lead to an increase in GFR and to polyuria. This could explain why eating and rumination, when the animals had calmed down and the salivary secretion had increased, inhibited the polyuria. However, its resemblance to water diuresis — for example the fact that 5 milliunits of "Pituitrin" inhibit the polyuria for about one hour — indicates that the state of emotion has in some way inhibited the release of antidiuretic hormone from the neurohypophysis.

The intravenous injections of adrenaline and ephedrine into the goats seem mostly, in opposition to what seems to be the case in man and dog (SMITH 1951), to cause an increased GFR and, in consequence, a polyuria. Thus the inulin clearance increased about 15 % after the intravenous injection of 5 cg of ephedrine. As an increased GFR means an increased load of electrolytes to the tubular reabsorptive system, this might explain the considerable rise in urinary excretion of Na^+ and Cl^- during the adrenaline and ephedrine polyuria in both groups of animals. In the saltdepleted animals, where urinary excretion of Cl^- and especially that of Na^+ was minimal before the injection of adrenaline and ephedrine, the rise in GFR probably increased the load of Na^+ and Cl^- well over the maximal rate of tubular reabsorption. This in turn might have caused the big increase in urinary concentrations of these electrolytes observed in the Group 2 animals. They did not, however, nearly reach their plasma levels.

The fact that, on the contrary, a decrease in U_{Na} and U_{Cl} towards the plasma levels was observed in the animals fed with free access to NaCl can probably be explained as follows. The more rapid passage of urine caused by increased GFR gives less

time available for the reabsorption of water during the transport through the distal tubuli.

The extensive urinary excretion of K^+ observed in the goats is explained by the fact that hay of the kind used to feed the animals contains about 20 g of K and only 1—2 g of Na per kg dry weight (CARLSTRÖM and CARLSTRÖM 1950). But, since potassium/inulin clearance ratios as high as 1 were not observed, the experiments do not give any proof of tubular excretion of K^+. If however, as has been suggested, an extensive reabsorption of K^+ occurs in the proximal tubule (SMITH 1951), it seems difficult to ascribe the large urinary excretion of potassium, found here, to anything but tubular excretion. The fact that the very big rise in the urinary excretion of Na^+ and Cl^- observed during the ephedrine polyuria was not accompanied by any similar rise in the excretion of K^+ seems to support this idea.

Summary.

A study was made of some features of the water and electrolyte metabolism of the goat.

1) Plasma Na^+ concentration was normally found within the range of 145—151 m Eq/l, plasma K^+ within 4—5 m Eq/l. Larger variations were found in the plasma Cl^- concentration.

2) Water up to 20% of their body weight was administered in a single dose on various occasions into the rumen of the goats without causing any signs of water intoxication.

3) The urinary excretion of such big doses of water took more than 13 hours, and the water diuresis remained during the first 6 hours at a fairly constant level.

4) 5—10 milliunits of "Pituitrin" was not found to have any obvious natriuretic or chloruretic effect on these animals.

5) During mild emotional stress the animals often developed a marked polyuria simultaneous with characteristic changes in their extracellular electrolyte pattern. This phenomenon was most frequently observed in goats on a low dietary intake of NaCl.

6) The intravenous injection of adrenaline (0.5 mg) and that of ephedrine (5 cg) caused a marked polyuria. A big rise in the urinary excretion of Na^+ and Cl^- and an increase of inulin clearance were observed during this polyuria.

7) The urinary excretion of Cl^- and especially that of Na^+

was extremely low in animals which had been fed for more than three months without access to extra NaCl. Thus U_K/U_{Na} ratios of up to 300 were found in these animals.

8) The rate of urinary excretion of K^+ was high and remained fairly constant during the experiments, but, as a consequence of variations in the rate of urine flow, variations of U_K/P_K ratios between 2 and 70 were observed. When potassium/inulin clearance ratios were measured these were within the range of 0.5—0.7.

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Note on the Influence of Hydrogen Ion Concentration Upon Swelling of Desheathed Peripheral Nerve.

By

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Desheathed peripheral nerve gradually increases in weight and volume when immersed in Ringer's solution, whereas a nerve with the connective tissue sheath left intact remains unchanged (FENG and LIU 1949, SHANES 1951, FRANKENHAEUSER and NYSTRÖM 1954). In order to find out whether the swelling of desheathed nerve is due to uptake of water by the nerve fibres or to accumulation of water between them, FRANKENHAEUSER and NYSTRÖM compared the behaviour of desheathed nerve, soaked in Ringer, with that of isolated fibres. They found that the nerve fibres themselves did not swell markedly, and concluded that the fluid taken up by the desheathed nerve was absorbed by the connective tissue or accumulated in interstitial spaces.

If the swelling is due to absorption of water by the connective tissue, it might be expected to be influenced by changes in hydrogen ion concentration, as is the case with proteins (LOEB 1922), and has been demonstrated with connective tissue fibres from muscle fascia of rat (DAY 1947 and 1949). In the present investigation the behaviour of desheathed nerves soaked in buffered Ringer of different pH values has been studied.

Technique.

The experiments were performed in the same way as those of FRANKENHAEUSER and NYSTRÖM on desheathed gastrocnemius nerve of frog (*Rana esculenta*). All dissections were carried out in air without any Ringer on the nerve. After the dissection the preparation was immersed in buffered Ringer, and its diameter was measured at intervals for half an hour. The diameter was measured in a dissecting microscope with an ocular micrometer (linear enlargement 100 times). In the buffered Ringer used the concentration of the different salts corresponded to the following molarity:

	mM
NaCl	63.0
KCl	2.5
$CaCl_2$	1.0
Sodium phosphate buffer	50.0

Results.

The results obtained show that the swelling of a desheathed peripheral frog nerve soaked in buffered Ringer's solution for half an hour has a minimal value at a pH of about 5 and increases when pH is shifted in any direction (Fig. 1).

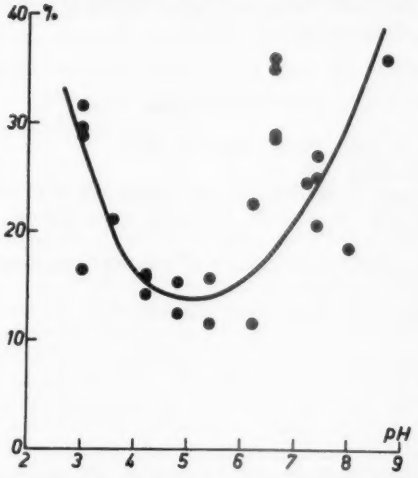

Fig. 1. Diameter changes of desheathed nerves in Ringer's solution of different pH values. Abscissa: pH of the solution. Ordinate: Diameter change after 30 min. soaking, expressed as per cent of the diameter after 1 min. soaking.

Discussion.

Our finding that the swelling of the nerve is markedly dependent upon the hydrogen ion concentration is consistent with the hypothesis that this swelling is due to absorption of water by the remaining connective tissue in the desheathed nerve. The minimal swelling at pH 5 indicates an isoelectric point at that pH. There seems, however, to be a peak of the values at pH 6.6, but the accuracy of our method does not permit us to draw any closer conclusions about the configuration of the curve. A few experiments with acetate instead of phosphate buffer gave higher values, but the results do not permit other conclusions than that there are at least quantitative differences between the anions used. This difference is not inconsistent with the hypothesis mentioned.

Summary.

The swelling of desheathed peripheral frog nerve, soaked in buffered Ringer's solution, is dependent on the pH of the solution and has a minimal value at a pH of about 5. This result is consistent with the hypothesis that the swelling is due to absorption of water by the connective tissue.

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Measurement of Total Body Water with Deuteriam Oxide and Antipyrine.

By

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At present deuterium oxide and antipyrine are considered the most suitable substances for the measurement of total body water. Deuterium oxide is thought to be an ideal substance, but expensive and the methods of determination are rather complicated. Antipyrine is more convenient with regard to these two factors and has thus been widely used.

The literature contains only limited data on the consistency of the measurement of total body water with the above substances. The aim of the present work was to evaluate the accuracy of the two methods of determination.

Material and Methods.

The measurements of the deuterium and antipyrine spaces were performed according to the principles given by SCHLOERB et al. (1950) and SOBERMAN et al. (1949) respectively.

The deuterium oxide (Norsk Hydro, Rjukan, Norway; 99.8 per cent) was transferred to dry bottles and then autoclaved. The bottles were weighed before and after pouring the deuterium into a infusion set filled with a small amount of saline. The difference indicated the weight of the deuterium. The mixture of deuterium oxide and saline was made isotonic with a few ml of 10 per cent NaCl. After a control sample of blood had been drawn, the deuterium oxide solution was given by intravenous infusion. The infusion set was carefully washed with saline

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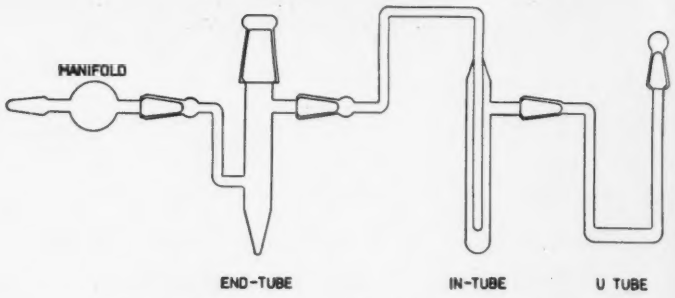

Fig. 1. Distillation train for deuterium oxide analyses.

while still attached to the vein. Exactly 50 ml of a 2.00 per cent solution of antipyrine was then infused from a calibrated burette. The connections were carefully flushed with saline. Venous blood samples were collected at about two and three hours after the infusions whereupon two more samples, at least one hour apart, were taken in the following five to six hours.

Pure plasma water was prepared by double distillation of the two hour and some of the three hour plasma samples (SCHLOERB et al. 1951). This was carried out in a Pyrex glassware distillation train, one unit of which is shown in Fig. 1. Fourteen such units were attached to a 14-outlet manifold, so that fourteen samples could be distilled simultaneously (Fig. 2). About 1/2 ml of the plasma samples and of suitable standards were introduced in the U-tubes (Fig. 1) and frozen down by brief immersion in a dry ice-alcohol mixture. By use of a vacuum-pump the samples were distilled to the in-tubes (Fig. 1), which were immersed in dry ice-alcohol. Dry air was admitted to the system, the samples melted, and the U-tubes were removed. The in-tubes were then immersed in water at about 30° C, and the samples slowly distilled to the end-tubes (Fig. 1) in a similar manner. The end-tubes were then removed and capped until the analyses were done.

The concentration of *deuterium* in the plasma water was determined by the gradient tube method (LINDERSTRÖM-LANG and LANTZ 1938, LINDERSTRÖM-LANG et al. 1938).

Gradient tubes were placed in a water bath at a temperature of 26.2° C, constant to $\pm$ 0.002° C. Suitable diffusion gradients at this temperature were obtained by mixing the following two solutions of kerosene and brombenzene: 1. a heavier solution consisting of 170.0 g of kerosone and 149.2 g of brombenzene; 2. a lighter solution consisting of 170.0 g of kerosene and 148.1 g of brombenzene. The kerosene used was white spirit (Nynäshamns Petroleum AB) and the brombenzene of a technical quality with the boiling point 154—158° C (Dow Chemical Co.). A linear gradient in the concentration range of 0.000—0.400 grams per cent of deuterium oxide was in general obtained three to four weeks after the preparation of the tubes. At this

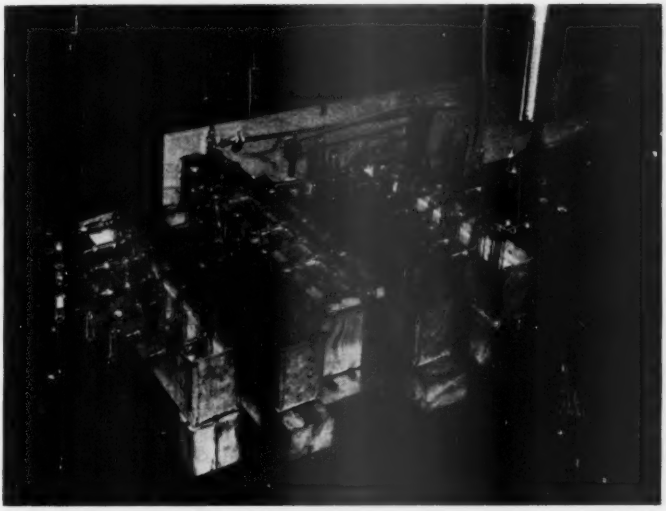

Fig. 2. The distillation apparatus joined to a central vacuum pump and manometer.

time, an increase in the deuterium oxide concentration of 0.100 grams per cent corresponded to a distance in the tube of about 15 mm. The tube could be used for two to three months, during which the margins of the gradient had become narrower, and 0.100 grams per cent of deuterium oxide corresponded to a distance less than 10 mm. The margin of error then became too great, and the tube had to be discarded.

Standard solutions, expressed as grams per cent (weight per weight), were prepared by mixing weighed amounts of deuterium oxide and triple distilled water. The weighings were performed to 0.1 mg on a microbalance. Five to six standard solutions between 0.000—0.350 grams per cent were prepared.

Each sample of plasma and standard was distilled in duplicate. Two drops of exactly the same magnitude were taken from each distillate and delivered into the tubes from a small, calibrated and siliconated capillary. Their position in the tube was determined to 0.05 mm with a measuring microscope (Cambridge Measuring Microscope; The Cambridge Instrument Co., London). The concentration of deuterium in the drops of plasma water was then determined from the position in the linear gradient of these drops and the drops of the standard solutions. The final value for the deuterium concentration was the mean of at least three such determinations.

The values for the plasma water concentrations of deuterium at equilibrium were corrected for the blank concentrations when repeated

measurements of the deuterium space were made. In accordance with the findings of SCHLOERB et al. (1950) the urinary excretion of deuterium during the equilibration time was taken as 0.4 per cent of the amount given. The deuterium space was then calculated as follows:

$$\text{deuterium space (litres)} = \frac{(100 - 0.4) \cdot D}{1000 \cdot d \cdot p},$$

where D denotes the amount of deuterium injected in grams, p the plasma concentration of deuterium at equilibrium in grams per cent corrected for the blank concentration, and d the density of H_2O at 37° C. (0.9934; CHANG and TUNG 1949).

The *antipyrine* of all the plasma samples was estimated according to the method of BRODIE et al. (1949) with a modification (IKKOS et al. 1954). The water content of plasma was taken as 93 per cent. The concentrations of the plasma samples were corrected for the blank concentration. The determination of the antipyrine space was then performed according to SOBERMAN et al. (1949). The fall of the plasma concentration of antipyrine was linear, when plotted semilogarithmically against time. The regression line was calculated, and the concentration at zero-time was then determined. The calculation of the antipyrine space was made as follows:

$$\text{antipyrine space (litres)} = \frac{AP}{p},$$

where AP denotes the amount of antipyrine injected in mg and p the corrected concentration of antipyrine in plasma water in mg per liter at zero-time.

Repeated simultaneous determinations of the deuterium and antipyrine spaces were performed in two healthy males, D. I. and H. L., 32 and 34 years old. Their body heights were 167 and 179 cm and the body weights 70 and 76 kg, respectively. The latter varied within one kg during the whole time of the study. No special attention was paid to the intake of food and fluid. The measurements were performed within two months, during which seven measurements were made on subject D. I. and eight on subject H. L.

In addition 16 non oedematous subjects were used for the determination of the equilibration time of deuterium. Twelve patients had various endocrine disorders which are listed in Table IV. The remaining four were normal.

Results.

I. Errors of the Methods.

a) *Errors in the determination of deuterium with the gradient tube method* (Table I).

The deuterium concentrations of known solutions of deuterium oxide in water were determined in 88 distilled and 139 non distilled samples by use of corresponding standards. The concentration

Table I.

Difference between known concentrations of deuterium oxide and the concentrations determined by the gradient tube method.

Distilled samples		Non distilled samples	
Difference grams per cent	No. of samples	Difference grams per cent	No. of samples
— 0.005	—	— 0.005	4
— 0.004	—	— 0.004	1
— 0.003	2	— 0.003	7
— 0.002	10	— 0.002	17
— 0.001	14	— 0.001	32
0.000	31	0.000	28
+ 0.001	15	+ 0.001	27
+ 0.002	8	+ 0.002	17
+ 0.003	5	+ 0.003	4
+ 0.004	3	+ 0.004	2
	total 88		total 139
Mean + 0.00020 (MD1)		— 0.00022 (MD2)	
S. D. ± 0.0015		± 0.0018	
S. E. ± 0.00016		± 0.00015	
P value		< 0.1, > 0.05	

ranges were 0.056—0.228 and 0.054—0.322 grams per cent, respectively. The mean differences, MD, between the concentrations known and the concentrations determined were + 0.00020 ± 0.00016 grams per cent for the distilled, MD1, and — 0.00022 ± 0.00015 grams per cent for the non distilled samples, MD2, respectively, the standard deviations being ± 0.0015 and ± 0.0018 grams per cent. There was no significant difference between the means, MD1 and MD2, the P-value being < 0.1, > 0.05. Combining the two groups, the mean difference, MD3, was — 0.00005 ± 0.00011 grams per cent, the standard deviation being ± 0.0017 grams per cent.

b) *Errors in the distillation procedure* (Table II).

The concentrations of known standards after distillation were determined with the use of non distilled standards. The differences between the concentrations known and the concentrations found were recorded. In one of these experiments the mean difference, — 0.0032 ± 0.0007 grams per cent, was larger than MD3, the P-value being < 0.001 (Table II). The result indicates that an error may be involved in the distillation procedure, manifested in this study by a decrease in the density of the samples.

Table II.

The concentration of deuterium oxide in known standards, determined after distillation.

Concentration known grams per cent	Concentration found grams per cent	Difference grams per cent
0.000	— 0.001	— 0.001
0.000	— 0.004	— 0.004
0.068	0.066	— 0.002
0.132	0.128	— 0.004
0.151	0.151	0.000
0.218	0.215	— 0.003
0.251	0.247	— 0.004
0.302	0.295	— 0.007
0.330	0.326	— 0.004
Mean		— 0.0032
S. D.		± 0.0021
S. E.		± 0.0007

Table III.

Recovery of deuterium added to plasma.

Sample		Concentration expected[1] grams per cent	Concentration found grams per cent	Recovery per cent
Standard, 0.559 grams per cent ml	Plasma ml			
1.00	1.00	0.290	0.288	99.3
			0.289	99.7
1.00	2.00	0.195	0.192	98.5
			0.194	99.5
2.00	1.00	0.382	0.379	99.2
			0.379	99.2

[1] The water content of plasma taken as 93 per cent by volume.

c) *Recovery of deuterium added to plasma* (Table III).

The recovery of deuterium added to plasma was measured. The water content of plasma was taken as 93 per cent by volume. Table III shows that the deuterium added could be recovered with a high degree of accuracy.

d) *Errors in the determination of antipyrine.*

Eighty-two duplicate analyses of plasma concentrations of antipyrine were made. The analytical error expressed as coefficient of variation was found to be ± 1.7 per cent.

Table IV.

Equilibration of deuterium oxide in the body. The concentration of deuterium oxide in plasma, two and three hours after the injection.

Subject		2 hrs sample grams per cent	3 hrs sample grams per cent	Difference grams per cent
D. I.	I	0.119	0.119	0.000
	II	0.204	0.204	0.000
H. L.	I	0.209	0.211	+ 0.002
	II (see text)	0.114	0.112	— 0.002
	III	0.191	0.190	— 0.001
	IV	0.248	0.245	— 0.003
	V	0.220	0.219	— 0.001
	VI	0.246	0.244	— 0.002
I. V.	normal	0.152	0.150	— 0.002
W. K.	normal	0.137	0.137	0.000
Å. S.	normal	0.111	0.110	— 0.001
R. P.	normal	0.112	0.112	0.000
G. A.	obesity	0.201	0.200	— 0.001
G. B.	obesity	0.167	0.168	+ 0.001
C. L.	obesity	0.081	0.081	0.000
A. A.	obesity	0.121	0.123	+ 0.002
I. J.	obesity	0.115	0.112	— 0.003
I. A.	anorexia	0.187	0.187	0.000
B. E.	diabetes	0.190	0.188	— 0.002
B. H.	acromegaly	0.176	0.176	0.000
A. G.	acromegaly	0.135	0.132	— 0.003
I. E.	acromegaly	0.124	0.124	0.000
J. J.	Cushing's syndrome	0.149	0.147	— 0.002
A. B.	hypophysectomy	0.094	0.093	— 0.001
Mean				— 0.00079
S. D.				± 0.0014
S. E.				± 0.00029

e) *Control of attained equilibrium* (Table IV, Fig. 3).

Table IV gives the values for the concentrations of deuterium in the 2 and 3 hour samples determined in subjects D. I. and H. L., as well as in 16 non oedematous subjects. The mean difference between the 2 and 3 hour values was -0.00079 ± 0.00029 grams per cent. Expressing the analytical error for each of the determined concentrations as the standard deviation of MD1, *i. e.* ± 0.0015 grams per cent, the analytical error for the mean difference between the 2 and 3 hour concentrations was found to be $\frac{\pm 0.0015 \times \sqrt{2}}{\sqrt{24}}$ grams per cent $= \pm 0.00043$ grams per cent.

Taking this error into consideration, the mean difference found was not significant, the P-value being $< 0.2 > 0.1$. Moreover, taking into account the biological half-time of deuterium, taken as 10 days and representing in this study an hourly decrease of 0.0005 grams per cent from the mean 2 hour value (0.158 grams per cent), the P-value for the difference increased to $< 0.6, > 0.5$. A constant concentration of deuterium in plasma was thus evi-

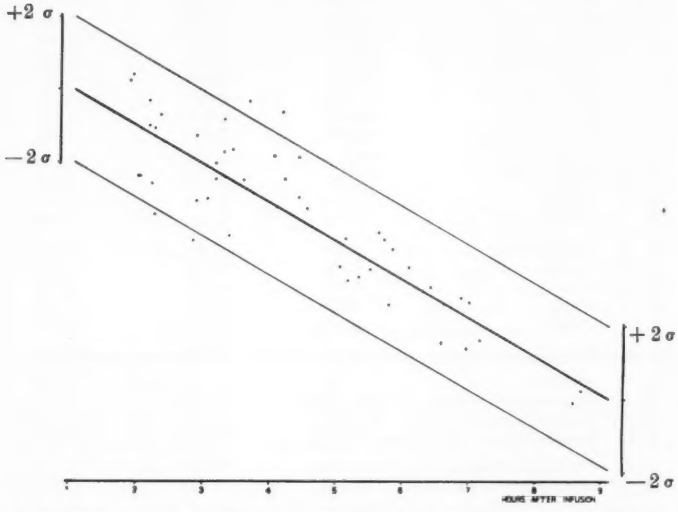

Fig. 3. Deviations of the antipyrine concentrations from the regression lines in 15 measurements of the antipyrine space in two healthy adults. The individual regression lines have been transferred to a common line. The 2 σ-limits for the analytical error of antipyrine is also given.

dent after two hours, confirming an even distribution of deuterium in the total body water (deuterium equilibrium). The 2 hour value was taken as the equilibrium concentration in the calculation of the deuterium space.

The antipyrine space was measured in subjects D. I. and H. L., and the deviations of the plasma concentrations of antipyrine from the calculated regression lines were determined. As may be seen from Fig. 3, the deviations — with four exceptions out of 60 — fell within the 2 σ-limits for the analytical error. Thus, within the accuracy of the analytical method, the fall of the plasma concentrations was linear.

Table V.

Error in the calculation of the zero-time concentration of antipyrine in plasma water.

Subject D. I.				Subject H. L.			
Date	Zero-time concentration mg per liter	Standard error mg per liter	Error in per cent of zero-time concentration	Date	Zero-time concentration mg per liter	Standard error mg per liter	Error in per cent of zero-time concentration
20.10	29.6	0	0	13.10	23.3	± 1.47	± 6.3
24.10	27.2	+ 2.58, — 2.40	+9.5,—8.8	16.10	23.5	± 1.29	± 5.5
1.11	26.7	+ 1.01, — 0.92	+3.8,—3.4	26.10	23.0	0	0
12.11	25.3	0	0	5.11	24.5	± 1.01	± 4.1
25.11	28.4	0	0	13.11	25.2	± 0.65	± 2.6
10.12	28.0	+ 0.74, — 0.65	+2.6,—2.3	30.11	23.7	± 1.57	± 6.6
16.12	29.2	+ 1.20, — 1.11	+4.1,—3.8	12.12	22.8	± 1.47	± 6.4
				19.12	25.9	± 1.38	± 5.3

f) *The error of the determination of the plasma concentration of deuterium at equilibrium.*

The error of determination of the plasma water concentration of deuterium may be expressed by the standard deviation of MD1, ± 0.0015 grams per cent. As the final values for the equilibrium concentrations were the means of at least three separate determinations, this error may be reduced by $1/\sqrt{3}$, *i. e.* to ± 0.0009 grams per cent. In the measurements of the deuterium space in the two subjects D. I. and H. L. the deuterium injected gave a mean increase of 0.122 grams per cent in the plasma concentration (Table VI). In these measurements, however, the total error of determination of the plasma concentration is composed of the error of the determination of the blank as well as the equilibrium concentration. This total error calculated from the standard deviation of MD1 and the mean increase in plasma concentration corresponds to a coefficient of variation of ± 1.0 per cent.

g) *The error of the zero-time concentration of antipyrine.*

The relation between time and antipyrine concentration, the latter logarithmically transformed, could be represented by a straight line. The equation for the regression line was calculated, and the standard errors of the zero-time concentrations were estimated applying the method of calculating the sampling error of regression constants (MATHER 1949). As shown in Table V

Table

The consistency of the measurements of the deuterium

Subject D. I.						
Date	Deuterium			Antipyrine		
	Injected grams	Equilibrium concentration grams per cent	Space litres	Injected mg	Zero-time concentration mg per litre	Space litres
20.10	46.45	0.119	39.1	1,000	29.6	33.8
24.10	46.93	0.116	40.6	1,000	27.2	36.8
1.11	51.56	0.132	39.2	1,000	26.7	37.4
12.11	50.69	0.128	39.8	1,000	25.3	39.6
25.11	75.44	0.186	40.5	1,000	28.4	35.2
10.12	43.73	0.113	38.8	1,000	28.0	35.7
16.12	45.58	0.114	40.1	1,000	29.2	34.2
Mean			39.73			36.10
S. D.			± 0.71			± 2.01
S. E.			± 0.27			± 0.82
Coeff. of variation			± 1.80			± 5.57

this error amounted to between 0.0—9.5 per cent of the zero-time concentration, the mean being 3.7 per cent. The difference in magnitude between the errors with positive and negative signs is explained by the logarithmic transformation of the antipyrine concentrations.

II. Measurements of Total Body Water.

The results of the measurements of total body water with the deuterium and the antipyrine methods are shown in Table VI. In subject D. I. the deuterium space varied between 38.8 and 40.6 litres, the mean being 39.7 ± 0.27 litres and the standard deviation ± 0.71 litres. The antipyrine space varied between 33.8 and 39.6 litres; the mean was 36.1 ± 0.82 litres and the standard deviation ± 2.01 litres. In subject H. L. the deuterium space varied between 44.4 and 47.2 litres, the mean being 45.8 ± 0.31 litres and the standard deviation ± 0.89 litres. The antipyrine space varied between 38.6 and 43.9 litres with a mean of 41.8 ± 0.66 litres and a standard deviation of ± 1.88 litres.

The consistency of the deuterium spaces, expressed by the coefficients of variation was ± 1.8 and ± 2.0 per cent. The

VI.

and the antipyrine spaces in two healthy subjects.

Subject H. L.						
Date	Deuterium			Antipyrine		
	Injected grams	Equilibrium concentration grams per cent	Space litres	Injected mg	Zero-time concentration mg per litre	Space litres
13.10	50.60	0.113	44.9	1,000	23.3	42.9
16.10	45.71	0.097	47.2	1,000	23.5	42.6
26.10	50.63	0.112	45.3	1,000	23.0	43.4
5.11	50.66	0.110	46.2	1,000	24.5	40.8
13.11	47.67	0.107	46.0	1,000	25.2	39.7
30.11	79.08	0.171	46.4	1,000	23.7	42.2
12.12	48.74	0.110	44.4	1,000	22.8	43.9
19.12	49.45	0.108	45.9	1,000	25.9	38.6
			45.79			41.76
			± 0.89			± 1.88
			± 0.31			± 0.66
			± 1.96			± 4.50

corresponding figures for the antipyrine spaces were ± 5.6 and 4.5 per cent.

The differences between the means of the deuterium spaces and the antipyrine spaces in the two subjects were 3.6 and 4.0 litres, corresponding to 9.1 and 8.7 per cent of the mean deuterium spaces.

Discussion.

The gradient tube method has been considered less accurate than the falling drop method for the determination of deuterium (Moore 1954). However, the error found in the present investigation, ± 0.0017 grams per cent,[1] corresponding to ± 0.0015 volumes per cent, is equal to that reported for the falling drop method by Schloerb et al. (1951). Moreover, Friis-Hansen (1954) recently reported an error of only ± 0.0008 volumes per cent with the gradient tube method. It thus seems that the accuracy of both methods is the same.

Contaminations of the samples during the distillation procedure may introduce errors in the determination of the density.

[1] MD3.

The sources of such errors have been discussed by SCHLOERB et al. (1951). In one of the present studies the determined concentrations of deuterium after distillation were lower than the initial ones. To avoid the influence of such errors the concentration of deuterium in plasma water was calculated with the use of standards distilled simultaneously with the plasma samples. The recovery obtained with this technique was highly satisfactory.

No significant difference in the analytical error could be demonstrated when the determinations were performed on distilled or non distilled standards.

The error of $\pm$ 1.7 per cent in the antipyrine analysis corresponds to that reported by BRODIE (1951) in recovery experiments and by KEYS and BROŽEK (1953) in duplicate determinations.

The variability of $\pm$ 1.8 and $\pm$ 2.0 per cent in the deuterium spaces in the two subjects studied, is in accordance with the error of two per cent for the single measurement suggested by SCHLOERB et al. (1951). The variability of the antipyrine spaces, $\pm$ 4.5 and 5.6 per cent, is in agreement with the findings of STEELE et al. (1950). As the total body water probably shows very small variation under normal conditions, the changes found may mainly be ascribed to errors in the measurements. An attained equilibrium could be shown for both the test substances. It may thus be presumed that the essential part of these errors was the analytical error of the equilibrium concentration of deuterium and the error of the calculated zero-time concentration of antipyrine. In this study the former was estimated to about one per cent while the latter varied between zero and ten per cent, the mean being 3.7 per cent. Consequently, the greater variability of the antipyrine spaces may be explained by this larger error. It was also pointed out by BRODIE (1951) that a higher reproducibility of the antipyrine space should not be expected with the errors involved in the determination of the zero-time concentration.

The deuterium spaces were found to be larger than the antipyrine spaces. This is in accordance with earlier findings (SOBERMAN et al. 1949, FRIIS-HANSEN et al. 1951, HURST et al. 1952). As deuterium exchanges with all labile hydrogen in the body it is probable that the deuterium space is overestimating the true volume of total body water. The magnitude of this error has

been estimated to one to two per cent of body weight (HEVESY and JACOBSEN 1940, SCHLOERB et al. 1950). On the other hand, it is probable that the antipyrine space is an underestimate of the true volume. It has been shown that antipyrine is bound to plasma protein (SOBERMAN et al. 1949) and the calculation of the antipyrine space is always made without any correction for this error. In animals, however, a very good agreement has been found between the deuterium as well as the antipyrine space and total body water determined by desiccation (MOORE 1946, SOBERMAN 1950). It is therefore still an open question, which of the two spaces more closely agrees with the true volume of total body water. In this respect each of the methods may probably be used with the same reliability. However, when changes in the volume are to be followed in the same subject during a period of time, the method with the highest consistency is preferable. The deuterium method seems to be the most suitable one for this purpose.

Summary.

The total body water of two healthy subjects was measured simultaneously with deuterium oxide and antipyrine. Within two months seven measurements were performed on one of the subjects and eight on the other. The variability of the deuterium spaces, expressed by the coefficient of variation, was 1.8 and 2.0 per cent. The variability of the antipyrine spaces was 4.5 and 5.4 per cent, respectively.

The analytical error of the equilibrium concentration of deuterium was estimated to about one per cent, whereas the average analytical error of the zero-time concentration of antipyrine amounted to about four per cent. The greater variability of the antipyrine space is probably due to this larger error.

The accuracy of the measurement of deuterium oxide concentration by the gradient tube method was investigated. The analytical error was found to be $\pm$ 0.0017 grams per cent.

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Sensitivity of the Normal and Denervated Parotid Gland to Chemical Agents.

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It has been shown for various structures that section of the efferent nerve renders the structure thus denervated more sensitive to drugs.

The accumulated evidence lead CANNON (1939) to formulate his law of denervation: "When in a series of efferent neurons a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures, the effects being maximal in the part directly denervated."

One of the organs the sensitization by denervation of which has been most extensively studied is the submaxillary gland (for references see EMMELIN 1952). In view of this it may seem unnecessary to investigate another salivary gland from this point of view. The innervation of the parotid gland, however, offers an opportunity to compare the effect of section of the ultimate and the penultimate parasympathetic nerve fibres, because the ganglion lies well outside the effector organ and is thereby an exception to the general rule of arrangement in the parasympathetic nervous system. There is another exception, the ciliary ganglion, and KEIL and ROOT (1941) made use of this in their study of the sensitization of the iris. These investigators found that section of the preganglionic fibres leading to the ciliary ganglion had a sensitizing effect approximately as big as that induced by removal of the ciliary ganglion as regards acetylcholine, the substance tried. As this statement is not in accordance with

the last statement in the law of denervation, it was thought to be of interest to compare the pre- and postganglionic denervation of the parotid gland.

The innervation of the parotid gland has been a matter of much dispute, but, at least in the cat and dog, the following facts seem to be established: the secretory fibres leave the brain with the IX. cranial nerve and arrive at the minor superficial petrosal nerve by way of the tympanic nerve (LOEB 1869, HEIDENHAIN 1878, BAYLISS and BRADFORD 1887). The minor superficial petrosal nerve carries the secretory fibres to the otic ganglion (BERNARD 1858, SCHIFF 1858, NAWROCKI 1868), from which they are conveyed by the auriculo-temporal nerve (SCHIFF 1858, NAWROCKI 1868) to the parotid gland. The impulses are synaptically transmitted through the otic ganglion (LANGLEY 1898). No nerve cells are to be found inside the gland itself (LANGLEY 1898).

Methods.

Forty-seven cats weighing 3—4 kg were used. The operations to be described were carried out under asepsis in nembutal anaesthesia given intraperitoneally (30—50 mg/kg). The preganglionic denervation (interruption of the tympanic nerve) was performed as follows:

A transverse wound 2—3 cms was made in the skin of the lateral part of the neck 3—4 cms below the outer acoustic meatus, thus avoiding damage to the parotid gland. The trapezoid muscle was split, and by blunt dissection the hind part of the digastric muscle was reached and split. The tympanic bulla now exposed could easily be trephined and the tympanic plexus (part of which is the tympanic nerve) was then carefully destroyed mechanically. The skin wound was sutured.

To reach the postganglionic fibres an incision (1—2 cms long) was made along the zygomatic process. The temporal muscle was exposed and then by blunt dissection along the outer aspect of this muscle the auriculo-temporal nerve was reached and a piece of the nerve about 5 mm long was excised. Half of the preganglionic denervations were carried out on the right, half on the left side; but this was at random. The same was true for the postganglionic denervations.

After a period of 12—16 days the cats were anaesthetized with chloralose (about 80 mg/kg intravenously after preliminary ether anaesthesia). This time period (12—16 days) was chosen since the sensitization of the submaxillary gland seems to have reached its maximum at this time (EMMELIN and MUREN 1952). A longer period was avoided as there is some evidence that the sensitization to acetylcholine is reduced with time (KEIL and ROOT 1941). The parotid ducts were exposed near the entrance in the mouth through a skin incision and small glass cannulae were inserted into them. The same pair of cannulae was used all the time, giving about the same number of drops

for distilled water (5 cc of distilled water gave 190—200 drops for each cannula). Acetylcholine, adrenaline and pilocarpine were injected into a cannula in the femoral vein and the falling drops of saliva were counted. The success of the operations was tested at the beginning of the experiments by electrical stimulation in the bulla. Further procedures will be given below.

Results.

Control Experiments.

To test the statement in the literature, that the secretory impulses are synaptically transmitted through the otic ganglion and that the denervations performed thus really could be regarded as pre- and postganglionic, hexamethonium was used in some experiments.

In three cats under chloralose anaesthesia hexamethonium was injected intravenously and the tympanic plexus was stimulated electrically. Doses of 1—2 mg/kg of hexamethonium chloride reduced considerably the response to a stimulus that earlier had evoked a rapid secretion. About one hour later, when the effect of the first dose had worn off, 5 mg/kg of hexamethonium was given, and was found to abolish completely the effect of stimulation. In order to ascertain that this complete abolishment was not due to an atropine-like effect of hexamethonium small doses of acetylcholine (1 μg/kg) were injected intravenously before and after the administration of the hexamethonium. No difference in the secretory response to acetylcholine was found. The effect of hexamethonium consequently seemed to be entirely ganglionic and it could be concluded that all the fibres of the tympanic nerve stimulated were preganglionic. Since the secretory response to stimulation of the auriculo-temporal fibres was not diminished by injection of 5 μg/kg of hexamethonium it was inferred that these fibres are postganglionic.

On application to the tympanic plexus of a stimulus, considerably stronger than that required to cause secretion at a maximal rate a very scanty secretion was in some cases observed after acute cutting of the auriculo-temporal nerve. This secretion was completely abolished by atropine (1 μg/kg intravenously). The effect was never observed in cats in which the auriculo-temporal nerve had been cut 2 weeks in advance. It seems reasonable to suppose this effect to be due to irradiation of the excessively strong stimulus to the postganglionic fibres.

Normal Glands.

Secretion was evoked in 10 unoperated cats, by injection of acetylcholine and in 9 out of these 10 by injection of adrenaline.

Acetylcholine. The threshold dose of acetylcholine was estimated by beginning with an injection of 1 μg/kg, a dose to which all glands tested responded. The doses were then halved successively until no further secretion could be obtained. After the threshold dose thus had been determined, standard doses of 1, 5, 10 and 20 μg/kg were given. The results of these studies in normal cats are given in table I.

Table I.

Threshold doses of acetylcholine expressed as 1, 0.5, 0.25, 0.125 and 0.063 μg/kg together with secretory responses in number of drops evoked by 1, 5, 10 and 20 μg/kg of acetylcholine in nonoperated animals.

Cat no.	Threshold dose		Number of drops							
			1 μg/kg		5 μg/kg		10 μg/kg		20 μg/kg	
	right	left	right	left	right	left	right	left	right	left
1	1	1	1/4	1/4	5/6	5/6	1 5/6	1 5/6	3 1/2	3 1/2
2	0.5	0.5	1 1/2	1 1/4	6 1/2	5 1/2	9 1/4	7	11 1/4	8 3/4
3	0.5	0.5	1/2	1/2	1 1/2	1 1/2	2 1/3	2 1/2	3 1/4	3
4	0.25	0.25	1/2	1/2	2 1/2	3	3	4	7	7
5	0.25	0.25	3/4	3/4	4 1/2	4 1/3	6 1/2	6 1/2	8 1/4	7 1/2
6	0.5	0.5	1/2	1/2	2 1/8	2 1/4	3 1/2	3 1/2	6	6 1/2
7	0.25	0.25	1/3	1/3	2 1/2	2 1/2	4 1/2	4 1/4	6 1/4	5 1/2
8	1	1	1/8	1/8	1/2	1/2	1	1	2 1/4	2 1/3
9	1	1	1/8	1/8	1/2	1/2	1/4	1/4	3/4	3/4
10	0.5	0.5	1/4	1/4	1 1/3	1 1/3	1 3/4	1 3/4	2 3/4	2 3/4
Mean value	0.58	0.58	0.48	0.46	2.28	2.23	3.39	3.26	5.13	4.76

Adrenaline. Adrenaline was tested in the same experiment, after acetylcholine, using doses of 1, 5, 10, 50, and 100 μg/kg and with 15 minutes between the doses. The experiments with adrenaline were complicated by the observation that this drug, besides vascular and secretory effects, seemed to exert an expelling action on the parotid gland. The following observations speak in favour of this conception. The first dose (1 μg/kg) often gave a drop of saliva, the next one (5 μg/kg) a smaller response and so on until the response again began to increase on raising the dose (this increase was first observed with 20—50 μg/kg). If, however, a fairly big dose of adrenaline was first given (*e. g.* 50 μg/kg) and

the testing with the series of doses was then done, no flow could be found with the small doses. A dose as big as 20—50 μg/kg was now found to be necessary to give a response; the effect then increased on raising the dose. If, on the other hand, acetylcholine (20 μg/kg) was then injected and the series of adrenaline doses was tested the initial results could again be obtained, even the small doses now causing a flow. This could be observed even if the series of doses was tried 10 minutes after the acetylcholine injection. If after an acetylcholine injection a standard dose of adrenaline was injected repeatedly the response was less the second than the first time. A third dose and subsequent doses caused the same response as after a previous 50 μg/kg dose of adrenaline and this response could be obtained repeatedly. There was another difference between this response to the series of small adrenaline doses after previous acetylcholine and the response after previous 50 μg/kg adrenaline. In the former type the whole response, amounting to about 1 drop irrespective of the dose, had a short duration, completed in a few seconds, whereas in the latter case several minutes of secretion were required to form a drop.

When the bigger doses of adrenaline were used (50 and 100 μg/kg), it could be observed that part of the drop hanging from the cannula was slowly aspirated. In this way the response could be lessened by 1/4 to 1/2 of a drop. No difference was found whether the cannulae were inserted near the gland end or near the mouth end of the duct. The phenomena mentioned above were first observed on normal glands and then on denervated glands in which the phenomena were even more pronounced. From the observations described above it seems reasonable to assume the effect of small doses of adrenaline after acetylcholine to be due to some kind of mechanical extrusion of saliva caused by the adrenaline. In order to avoid these phenomena a 50 μg/kg dose of adrenaline was given half an hour before the secretory effect of adrenaline was studied. Between the different doses of adrenaline given there was an interval of 15 minutes. This time was chosen since the back flow of saliva mentioned was found to occur well within this period. It was further found that if a shorter interval than 15 minutes was used the response to repeated 50 μg/kg doses of adrenaline tended to diminish, an effect that might be due to vascular adjustments. The threshold doses for adrenaline and the secretory response to 50 μg/kg are shown in table II.

Table II.

Threshold doses of adrenaline expressed as 1, 5, 10, 20, 50, 100 or 150 µg/kg of adrenaline and the secretory responses in number of drops to 50 µg/kg of adrenaline in nonoperated animals.

Cat no.	Threshold dose		Number of drops 50 µg/kg	
	right	left	right	left
1.....	50	50	1/8	1/4
2.....	50	50	1/4	1/8
3.....	100	100	—	—
4.....	100	100	—	—
5.....	20	20	1/3	1/4
6.....	150	150	—	—
7.....	20	20	1	2/3
8.....	—	—	—	—
9.....	50	50	1/8	1/8
10.....	50	50	1/4	1/4
Mean value	66.67	66.67	0.23	0.19

Sensitization of the gland by denervation.

According to CANNON and ROSENBLUETH (1949) the term supersensitivity covers several phenomena. Thus the response to a certain stimulus may be greater in amplitude or longer lasting in the denervated than in a normally innervated structure. The denervation may in addition cause a lowering of the threshold of stimulation. In the case of salivary glands the first two qualities mentioned, magnitude and duration, may be taken together and expressed as number of drops of saliva evoked by the stimulus. This view has also been adopted by EMMELIN and MUREN (1951) in their work on the submaxillary gland.

In the experiments on normal cats it was found (tables I and II) that the dose of the drugs necessary to give a secretory response was the same on the two sides, but that the threshold dose showed individual variations. The same was true for moderate and bigger doses. Because of the differences found between animals it was thought preferable to gain information by comparison between the two glands of one and the same cat. Secretion was evoked by injection of acetylcholine, adrenaline and pilocarpine and by stimulation of the sympathetic nerve in the neck.

Acetylcholine. The results obtained in the studies of the threshold doses are given in table III.

From the table it can be deduced that when preganglionically denervated glands (in the following referred to as preglands) were

Table III.

Threshold doses of acetylcholine expressed as 1, 0.5, 0.25, 0.125, 0.063 μg/kg in cats variously treated.

Cat no.	Pre-gland	Normal gland	Cat no.	Post-gland	Normal gland	Cat no.	Pre-gland	Post-gland
14	0,125	0.5	11	0.25	1	18	0.125	0.125
17	0.125	0.125	12	0.125	0.5	20	0.25	0.125
22	0.25	1	13	0.25	1	21	0.5	0.5
23	0.125	0.5	15	0.125	1	27	0.125	0.125
24	1	1	16	0.125	1	29	0.125	0.125
25	0.125	0.25	19	0.5	1	30	0.25	0.25
26	0.125	0.25	28	0.125	0.5	31	0.25	0.125
34	0.25	0.5				32	0.125	0.063
35	0.5	0.5				33	0.063	0.063
36	0.25	0.5				37	0.5	0.25
38	0.25	0.25						
39	0.125	0.125						

compared with the contralateral, normally innervated glands (for brevity called normal glands) (12 cases), preglands were found to have a lower threshold in 8 cases and in the remaining 4 the thresholds were equal. Postganglionically denervated glands (postglands) compared with normal glands (7 cases) always had lower thresholds. Postglands compared with preglands (10 cases) had a lower threshold in 4 cases and in the remaining 6 cases the thresholds were the same. The mean value of the thresholds in this series of cats was for the normal glands 0.61 ± 0.074 (19) μg/kg; for the preglands 0.25 ± 0.045 (22) μg/kg, and for the postglands 0.19 ± 0.030 (17) μg/kg. There is high statistical probability that the normal threshold is higher than pregland threshold ($P < 0.001$) and that pregland threshold is higher than postgland threshold ($P < 0.01$).

As mentioned above a sensitization may be detected by comparing the number of drops evoked by a given stimulus. When acetylcholine was tried, the following standard doses were chosen: 1, 5, 10 and 20 μg/kg. The results obtained are shown in table IV.

A convenient index of sensitization would be the figure obtained by dividing the number of drops from one kind of gland with the number of drops from another kind of gland evoked by a standard dose in the same cat *e. g.* $\frac{\text{pregland response}}{\text{normal gland response}}$ to 1 μg/kg. If there were no difference between the glands variously treated the index would be about 1, but if one gland was sensitized in relation to its fellow gland the index would depart from 1.

Table IV.

The secretory responses in number of drops evoked by 1, 5, 10, and 20 µg/kg of acetylcholine from parotid glands variously treated.

Cat no.	1 µg/kg			5 µg/kg			10 µg/kg			20 µg/kg		
	Pre-gland	Post-gland	Norm. gland	Pre-gland	Post-gland	Norm. gland	Pre-gland	Post-gland	Norm. gland	Pre-gland	Post-gland	Norm. gland
11		5/6	1/8		3 1/3	1 1/3		6	2 1/2		7 1/2	3 1/2
12		2 1/2	5/6		4 1/2	1 5/6		8 1/3	3 1/2		9 1/2	4 5/6
13		5/6	1/4		2 1/3	5/6		3 1/2	1 5/6		4 1/2	3 1/4
14	1 1/4		1/3	2 5/6		2 1/2	3 3/4		5 1/2	5 1/2		8 1/2
15		1 1/2	1/4		1 5/6	1 1/4						
16		3/4	1/4		1 3/4	1 1/4		3 1/2	2 1/2		4 1/2	3 1/2
17	2 1/2		1 1/2	4		2 3/4						
18	1	2 1/4		3 1/4	6 1/4		6	10		9	13	
19		1/2	1/4		5/6	1/2		13/4	1		22/3	13/4
20	1 1/4	1 5/6		2 1/3	3 2/3		4 1/2	6 1/2		7	11 1/2	
21	1 1/8	1/2		4	3		6 1/2	4		7	6	
22	1/2		1/4	2		1 1/2	4 1/3		3 1/3	8		6 1/2
23	3/4		1/2	2 1/4		1 1/8	3 1/2		2 1/2	5 1/4		5 1/4
24	1/4		1/8	5/6		5/6	1 1/4		1 1/4	3 1/4		3 1/4
25	2/3		2/3	3 2/3		2 3/4	8 1/2		6 3/4	8 1/2		7 1/2
26	1 2/3		1 1/4	4 1/2		4 1/2	5 2/3		6 1/2	8		10 1/4
27	1 1/2	2 3/4		2 1/2	4 1/2		5 1/2	10 1/4		7 1/4	12 1/2	
28		1 3/4	1/2		5 2/3	1 1/2		10 2/3	4	10 1/2		4 2/3
29	2 1/2	4 1/2		5 1/2	12 1/2		7	16		13	20	
30	1/3	1/2		2 1/2	4 1/2		5	9		6 1/2	11 1/2	
31	1	1 1/2		4 1/2	6		7 1/2	10 1/2		12	14 1/2	
32	2 3/4	6 1/3		8	13 2/3		11 1/2	14 1/2		12 1/2	14 1/3	
33	3/4	1 2/3		6 1/2	8 3/4		8 1/2	10 3/4		9	11 1/4	
34	2/3		1/2	5		4	7 1/2		8	13 1/4		13 2/3
35	1/4		1/4	1 3/4		1 3/4	2 3/4		2 3/4	5		5
36	5/6		5/6	3 1/4		2 1/2	6		5	7		6
37	1/3	2/3		1 2/3	2 1/2		2 1/3	3 2/3		4	7	
38	5/6		5/6	6		6	9		8			
39	1 1/2		1 1/2	3 1/2		5 1/3	5		9 1/2	8		12
Mean value	1.10	1.83	0.58	3.65	5.03	2.32	5.79	8.06	4.38	7.98	10.05	6.21

The mean values of the indices for the standard doses together with the statistical probability indicating that the index in question differs from 1 $\pm$ the normal variation, are given in table V.

The normal variation between the sides for the various doses was calculated in the following way: In the experimental groups the denervations were done either on the left or on the right side. Thus the indices are either left to right or the reverse. In each case the experimental gland was chosen randomly. Therefore the indices obtained from untreated controls were also calculated

Table V.

The mean values of the indices (described in the text) for various doses of acetylcholin.

The significance that the index departs from 1 is indicated as follows:
*** = $P < 0.001$
** = $P < 0.01$
* = $P < 0.05$

	Pregland / Normal gland index	Postgland / Normal gland index	Postgland / Pregland index
1 μg/kg	1.55*	3.93***	1.73**
5 μg/kg	1.20*	2.30**	1.60***
10 μg/kg	1.03	2.08***	1.52**
20 μg/kg	0.96	1.76***	1.43***

as left to right or the reverse and the choice of this was determined by random numbers from the table of FISHER and YATES (1953). The values given indicate that the bigger the dose the less the relative difference between preglands, postglands and normal glands. This is quite in accordance with the results reported by EMMELIN and MUREN (1951) for the submaxillary gland.

Values hitherto given represent, as pointed out earlier, the combined effect of the secretory rate and the duration of secretion. The duration of secretion was always found to be longer in sensitized glands. To investigate whether the secretory "rate" was changed in a denervated gland, one might use a continuous stimulus causing secretion from both and record the time taken to form a drop. As a relatively continuous stimulus pilocarpine was chosen.

Pilocarpine. Pilocarpine was injected in repeated 10 μg/kg doses until 100 μg/kg had been given; then 50 μg/kg doses were used. The injections were given with intervals of 5 minutes. The time required to form 2 drops was measured by a stop watch and thus the secretory rate could be evaluated.

This type of experiment was performed in 8 cats. In general it was found that for moderate doses (up to a total dose of 150 μg/kg) a postgland secreted faster than a pregland and a pregland faster than a normal gland. The difference was less the bigger the dose. Maximal secretory rate was obtained by total doses of between 100 and 600 μg/kg. This rate varied in different cats between 2 drops in 3, 2 sec. and 2 drops in 20 sec. Sometimes a normal, sometimes a denervated gland was found to be able to give the quickest flow of saliva.

Table VI.

Threshold doses of adrenaline expressed as 1, 5, 10, 20, 50, and 100 μg/kg in cats variously treated.

Cat no.	Pre-gland	Norm. gland	Cat no.	Post-gland	Norm. gland	Cat no.	Pre-gland	Post-gland
14	5	10	11	20	50	18	5	5
17	20	20	13	10	20	20	1	5
23	5	50	15	5	10	21	5	50
24	10	50	16	5	20	27	10	10
25	20	100	28	10	20	30	20	50
26	20	50	40	5	50	31	100	100
34	20	20				32	10	10
35	10	20				33	5	5
36	10	100				37	10	20
38	1	10						
39	5	50						

Adrenaline. The threshold doses for adrenaline estimated in the way described for normal glands are found in table VI. Direct comparison between normal and preglands (11 cases) showed in 9 cases a lower threshold for the preglands; in the remaining two the thresholds were equal.

Postgland—normal gland comparison showed the postgland threshold to be the lower one in all six cases. Pre- and postgland comparison (9 cases) showed the pregland to be the one with the lowest threshold in 4 cases; the two kinds of glands had the same threshold in the remaining 5 cases. The mean values for the thresholds were: normal glands: 38 ± 6.8 μg/kg; preglands: 15 ± 4.8 μg/kg; and postglands: 21 ± 6.9 μg/kg. When the effects of 50 μg/kg doses were compared, this dose being chosen as the smallest one giving a measurable secretion from most of the glands, it was found that a considerable sensitization could be detected in denervated glands. This can be seen in table VII. The figures for the indices $\frac{\text{pregland}}{\text{normal gland}}$ and $\frac{\text{postgland}}{\text{normal gland}}$ are very high. It is thus obvious that both types of denervation cause a marked sensitization to adrenaline. The values for the $\frac{\text{postgland}}{\text{pregland}}$ indices show that in 5 out of 8 cases the sensitization by preganglionic denervation was greater than by postganglionic denervation; in one case the reverse was true, and 2 cases revealed the same secretory value; index: 1.

In two cats with pregland—normal glands dl-noradrenaline was

Table VII.

The secretory responses in number of drops evoked by 50 µg/kg of adrenaline together with the indices (described in text).

Cat no.	Pre-gland	Norm. gland	Pre-gland / Norm. gland index	Cat no.	Post-gland	Norm. gland	Post-gland / Norm. gland index	Cat no.	Pre-gland	Post-gland	Post-gland / Pre-gland index
14	2 1/2	1	2.50	11	5/6	1/8	6.67	18	1/3	2/3	2.00
17	7	5/6	8.40	13	1 1/4	1/3	3.75	20	3 1/4	1/2	0.15
23	1 5/6	1/6	11.00	15	2 1/4	1/4	9.00	21	1 1/2	1/8	0.08
24	3 1/6	1/3	9.50	16	2 1/2	1	2.50	27	1 1/4	2/3	0.53
25	1 1/4	0	∞	28	3 1/2	1/3	10.50	30	1/2	1/8	0.25
26	1/3	1/8	2.67					32	1/2	1/2	1.00
34	1/2	1/8	4.00					33	3/4	3/4	1.00
35	1/2	1/4	2.00					37	3/4	1/4	0.33
36	1/3	0	∞								

tested. The results with this drug were very similar to those obtained with adrenaline.

Sympathetic stimulation. In seven of the cats the sympathetic chain in the neck was stimulated electrically. On stimulation of the sympathetic nerve in the neck the parotid gland was found to respond, after a latency of some seconds, with a slow secretion. The rate of flow decreased on continued stimulation just as is the case with the submaxillary gland. If the stimulation was carried out some time after acetylcholine (20 µg/kg) was given, it was found that about one drop was formed rapidly and then the secretory rate decreased very much. Stimulation when adrenaline (50 µg/kg) had been injected earlier did not evoke this first rapid response. In parasympathetically decentralized glands sympathetic stimulation caused a more rapid secretion than in the contralateral control glands. The interval between the two first drops formed was 2—3 minutes in denervated, and about 10 minutes in normal glands. The difference between pre- and postglands was, if any, slight and was not evaluated.

Blood pressure effects of parotid saliva. Numerous investigators have demonstrated a potent depressor factor in the saliva secreted from the submaxillary gland. The most extensive study is that by Feldberg and Guimarais (1935). These investigators found that the depressor activity was much greater in the saliva obtained by stimulation of the sympathetic nerve than that obtained by stimulation of the chorda.

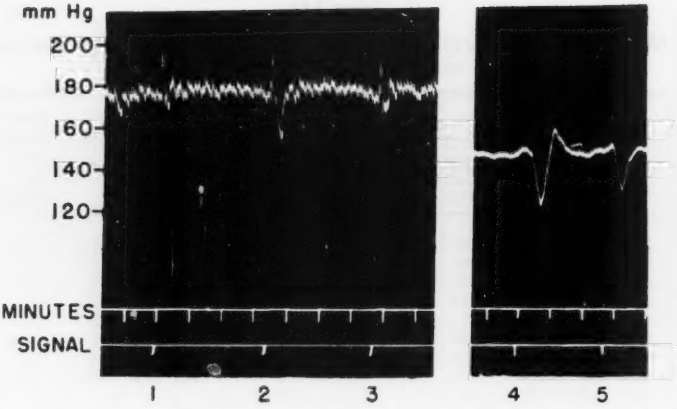

Fig. 1. Depressor activity of parotid saliva from two cats. First section of tracing shows a comparison between sympathetic and parasympathetic saliva in a 2.2 kg cat. Second section of tracing shows a comparison between adrenaline and acetylcholine saliva in a 1.7 kg cat. Time in minutes.

1 = Saline solution.
2 = 0.003 cc sympathetic saliva.
3 = 0.3 cc parasympathetic saliva.
4 = 0.001 cc adrenaline saliva.
5 = 0.05 cc acetylcholine saliva.

If the same difference between sympathetic and parasympathetic saliva was found for the parotid it might support the view that the scanty flow obtained by adrenaline and sympathetic stimulation was not altogether due to a mechanical extrusion. Experiments of this type were performed with saliva obtained from the nonoperated side of seven cats. In two of the cats the saliva was obtained by stimulation of the sympathetic in the neck and the tympanic plexus. From the five remaining cats saliva was obtained by injection of acetylcholine and adrenaline.

As the depressor activity seems to diminish when secretion is going on (FELDBERG and GUIMARAIS) the samples collected were of the same volume (about 1/2 cc). For the same reason the sympathetic saliva was collected first in one and the parasympathetic saliva first in the other of the two glands. When collecting acetylcholine-adrenaline saliva the same precautions were taken. To avoid the possible influence of the secretory rate on the content of the blood pressure active substances the parasympathetic stimulation was very weak and the doses of acetylcholine small so as to give a secretory rate which did not differ very much from

that obtained by sympathetic stimulation and adrenaline injections. The various samples were, after having been stored in refrigerator over night, injected intravenously into small cats, the blood pressure being recorded in the carotid artery. It was found that the saliva obtained by adrenaline injection or by sympathetic stimulation was very active in depressing the blood pressure, whereas the saliva obtained by acetylcholine or stimulation of the tympanic plexus was much less potent. The latter samples had sometimes a combined pressor-depressor effect well-known for submaxillary saliva. An amount as small as 0.001 cc of adrenaline or sympathetic saliva was usually enough to give a detectable fall in blood pressure. To get a detectable effect by acetylcholine or parasympathetic saliva 0.05 cc was usually necessary (Fig. 1).

Sympathetic denervation. The effect of sympathetic denervation was examined in 4 cats. In two of the cats the sympathetic nerve was cut, and in the others the superior cervical ganglion was excised. The sensitivity of the glands to adrenaline and acetylcholine was studied two weeks later. In two of these cats, one of each kind, a slight sensitization both to acetylcholine and adrenaline could be detected, but in the other two cats the operations seemed not to have influenced the sensitivity at all.

Discussion.

The only communication, to my knowledge, dealing with the action of sialogogue drugs on the parotid gland of the cat, is that of LANGLEY (1901) on the action of suprarenal extracts, in which it is stated that such extracts cause a lively secretion from all the salivary glands.

Adrenaline was in the present experiments found to evoke a flow of saliva, but the secretory effect was remarkably small when compared with that on the submaxillary gland. The results of this investigation seem to indicate that adrenaline has, apart from the vascular effect, a dual effect on the parotid gland of the cat: one expelling and one secretory. The backflow of saliva after big doses of adrenaline suggests that the saliva at least partly has been extruded mechanically. The effect of small doses of adrenaline, after acetylcholine had been injected previously, might likewise be explained by assuming a contractile mechanism. The structure responsible for this contraction might be relaxed by acetylcholine and thus more responsive to the action of an agent inducing

contraction (adrenaline) of the structure. In accordance with this is the fact that no backflow after the small doses of adrenaline used in this type of experiment could be detected; these doses only having caused a return of the contractile structure to the normal state from the relaxation caused by acetylcholine.

Big doses of adrenaline seem to evoke a real secretion. This statement is supported by the fact that the responses could be repeated, and is further suggested by the results obtained when the blood pressure effect of parotid saliva was estimated. The saliva collected after repeated injections of adrenaline differed markedly as regards the blood pressure action from the saliva obtained by injections of acetylcholine.

STAVRAKY (1931) experimenting on the parotid gland of the dog concluded histamine to have a dual action similar to that discussed here for adrenaline.

Like adrenaline, sympathetic stimulation was found in the present investigation to evoke only a scanty secretion. Earlier experiments on the secretory effect of sympathetic stimulation on the parotid gland of the cat have given controversial results. In his work "Parotis und Sympathicus" (1867) WITTICH denies that any secretion can be evoked by sympathetic stimulation. NAWROCKI (1868), on the other hand, found a slight secretion from the parotid gland of the cat, when stimulating the sympathetic. BAYLISS and BRADFORD (1887) report in a footnote: "In the parotid of the cat, as well as in the submaxillary, excitation of the sympathetic causes a copious watery secretion." BABKIN (1950) makes the general remark that the presence of secretory fibres in the sympathetic to the parotid gland is not definitely proved. The results obtained in the present investigation seem to agree with those of NAWROCKI (1868). In a recent work RICHINS and KUNTZ (1953) report a scanty flow of saliva from the parotid gland of the cat when stimulating the sympathetic chain in the neck.

As a further argument in favour of the opinion that sympathetic stimulation really gives rise to secretion may be mentioned the finding that saliva obtained in this way had a strong depressor activity, similar to that of adrenaline saliva. Parasympathetic saliva from the parotid gland was much less potent in lowering the blood pressure, and the saliva obtained by sympathetic stimulation can therefore not solely be expelled parasympathetic saliva.

An expelling action of the sympathetic similar to that of adrenaline is also suggested by the results given. The action on the parotid

gland would thus be very similar to that on the submaxillary gland as described by BABKIN and MACKAY (1930).

The main theme of this investigation has been the sensitization by denervation of the parotid gland of the cat. The results, obtained with the methods described in this paper, show that the parasympathetically denervated gland is more sensitive to acetylcholine, adrenaline and pilocarpine than the normally innervated gland.

The three principal components of sensitization: the lower threshold, the longer lasting action and the greater secretory rate were all found to exist for the sensitized parotid gland. The sensitization to adrenaline seemed to be greater than that to acetylcholine, a fact that was also found by EMMELIN and MUREN (1951) on the submaxillary gland.

Comparison between the effect of pre- and postganglionic denervations showed the postganglionic severance to be more effective in creating a supersensitivity to acetylcholine and pilocarpine. This is in agreement with the "Law of denervation", and differs from the results of KEIL and ROOT (1941) obtained with acetylcholine on another structure, the iris of the cat. The secretion in response to adrenaline was usually found to be greater from a pre- than from a postganglionically denervated gland. This might mean either that the mechanism responsible for the sensitization of the secretory elements to adrenaline is, at least partly, another than that responsible for the sensitization to acetylcholine and pilocarpine, or that some other factor is operating conjunctionally. Such a factor may be found in the circulatory changes in the gland induced by the denervation. A constriction induced by adrenaline could perhaps reduce the response from the postglands more than from the preglands if the vessels behave according to the "Law of denervation". To throw light on this discrepancy between adrenaline and the other substances used, studies on the circulation of the normal and the denervated and decentralized gland seem desirable.

Summary.

1. Experiments are described which show that parasympathetic denervation of the parotid gland renders the gland more sensitive to the secretory action of acetylcholine, adrenaline and pilocarpine.

2. Postganglionic denervation induces a greater sensitization

than preganglionic denervation as regards acetylcholine and pilocarpine. The reverse seems to be true for adrenaline.

3. Some evidence is given to show that sympathetic stimulation and injection of adrenaline has dual action on the parotid gland of the cat, apart from that on the vessels, one secretory and one expelling.

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